JC07 Rec'd PCT/PTO 05 NOV 2001

FORM PTO 1390 (Modified) (REV 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

# TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: 14445-504

U.S. APPLICATION NO (IF KNOWN, SEE 37 CFR): 10/009122

| INTERNATIONAL APPLICATION NO | INTERNATIONAL FILING DATE | PRIORITY DATE CLAIMED |
|---|---|---|
| PCT/CA00/00515 | 04 May 2000 (04.05.00) | 05 May 1999 (05.05.99) |

TITLE OF INVENTION

**STEREOSELECTIVE ANTIFIBRILLOGENIC PEPTIDES AND PEPTIDOMIMETICS THEREOF**

APPLICANT(S) FOR DO/EO/US

**CHALIFOUR, Robert; GERVAIS, Francine; GUPTA, Ajay**

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (24) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☐ has been communicated by the International Bureau
   c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☐ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A copy of the International Search Report (PCT/ISA/210).

**Items 13 to 20 below concern document(s) or information included:**

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification
18. ☐ A change of power of attorney and/or address letter.
19. ☒ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☒ Certificate of Mailing by Express Mail
23. ☒ Other items or information:

**Article 34 Amendments included in IPER**
**Limited Recognition under 37 CFR § 10.9(b)**
**Express Mail Label No: EL390884416US**
**Filed On: 05 November 2001 (05.11.01)**

| U.S. APPLICATION NO (IF KNOWN, SEE 37 CFR) | INTERNATIONAL APPLICATION NO | ATTORNEY'S DOCKET NUMBER |
|---|---|---|
| 10/009122 | PCT/CA00/00515 | 14445-504 |

24) The following fees are submitted:

**BASIC NATIONAL FEE ( 37 CFR 1.492 (a) (1) - (5)) :**

| | | CALCULATIONS PTO USE ONLY |
|---|---|---|
| ☐ | Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO . . . . . **$1000.00** | |
| ☒ | International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO . **$860.00** | |
| ☐ | International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1 445(a)(2)) paid to USPTO . . . . . **$710.00** | |
| ☐ | International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) . **$690.00** | |
| ☐ | International preliminary examination fee (37 CFR 1 482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) . . . **$100.00** | |

| | | |
|---|---|---|
| **ENTER APPROPRIATE BASIC FEE AMOUNT =** | **$860.00** | |
| Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)). | **$130.00** | |

| CLAIMS | NUMBER FILED | NUMBER EXTRA | RATE | | |
|---|---|---|---|---|---|
| Total claims | 29 - 20 = | 9 | x $18.00 | **$162.00** | |
| Independent claims | 2 - 3 = | 0 | x $80.00 | **$0.00** | |
| Multiple Dependent Claims **(check if applicable)**. | | | ☐ | **$0.00** | |
| **TOTAL OF ABOVE CALCULATIONS =** | | | | **$1,152.00** | |
| ☐ Applicant claims small entity status (See 37 CFR 1.27). The fees indicated above are reduced by 1/2 | | | | **$0.00** | |
| **SUBTOTAL =** | | | | **$1,152.00** | |
| Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). + | | | | **$0.00** | |
| **TOTAL NATIONAL FEE =** | | | | **$1,152.00** | |
| Fee for recording the enclosed assignment (37 CFR 1 21(h)) The assignment must be accompanied by an appropriate cover sheet (37 CFR 3 28, 3.31) **(check if applicable).** ☐ | | | | **$0.00** | |
| **TOTAL FEES ENCLOSED =** | | | | **$1,152.00** | |
| | | | | **Amount to be: refunded** | $ |
| | | | | **charged** | $ |

a. ☒ A check in the amount of **$1,152.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees
A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **50-0311** A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

**NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.**

SEND ALL CORRESPONDENCE TO:

**ELRIFI, Ivor R.**
**Mintz, Levin, Cohn, Ferris, Glovsky & Popeo, P.C.**
**One Financial Center**
**Boston, Massachusetts 02111**
**United States of America**

SIGNATURE

**MORENCY, Michel**
NAME

**Ltd Recognition**
REGISTRATION NUMBER

**05 November 2001 (05.11.01)**
DATE

10/009122
JC19 Rec'd PCT/PTO 05 NOV 2001

**Express Mail Label No.: EL 390884416US**
**Date of Deposit: November 5, 2001**

**Attorney Docket No. 14445-504 NATL**
**(NCHEM-4 NATL)**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

| | | | |
|---|---|---|---|
| APPLICANT(S): | Chalifour, *et al.* | | |
| SERIAL NUMBER: | Not Yet Assigned | EXAMINER: | Not Yet Assigned |
| FILING DATE: | November 5, 2001 | ART UNIT: | Not Yet Assigned |
| FOR: | *STEROSELECTIVE ANTIFIBRILLOGENIC PEPTIDES AND PEPTIDOMIMETICS THEREOF* | | |

November 5, 2001
Boston, Massachusetts

Commissioner for Patents
Washington, D.C. 20231

**PRELIMINARY AMENDMENT**

Please amend this application as follows:

***In the Claims:***

Please cancel claims 23-28, 30, 31, and 33 without prejudice or disclaimer and add new claims 37 and 38. Please amend the claims as follows:

1. (amended once) An antifibrillogenic agent for inhibiting amyloidosis and/or for cytoprotection, which comprises a peptide of Formula I, an isomer thereof, a retro or a retro-inverso isomer thereof or a peptidomimetic thereof:

$$Xaa_1\text{-}Xaa_2\text{-}Xaa_3\text{-}Xaa_4 \qquad I$$

wherein,

$Xaa_1$ is selected from the group consisting of Lys and $Xaa_5$-Lys-;

$Xaa_5$ is selected from the group consisting of Lys, His-Gln-, His-His-Gln-, Val-His-His-Gln-, Glu-Val-His-His-Gln-, Asp-Asp-Asp-, and Gln-;

$Xaa_2$ is any amino acid;

$Xaa_3$ is Val;

$Xaa_4$ is selected from the group consisting of Phe, Phe-$NH_2$, Phe-Phe, Phe-Phe-Ala, Phe-Phe-Ala-$NH_2$, Phe-Phe-Ala-Gln, and Phe-Phe-Ala-Gln-$NH_2$;

wherein said peptide has at least one [D] amino acid residue,

with the proviso that Lys-Lys-Leu-Val-Phe-Phe-Ala is an all-[D] peptide.

7. (amended once) The antifibrillogenic agent of claim 1, wherein said peptide of Formula I is selected from the group consisting of:

| | |
|---|---|
| Lys-Ile-Val-Phe-Phe-Ala | (SEQ ID NO:1); |
| Lys-Lys-Leu-Val-Phe-Phe-Ala | (SEQ ID NO:2); |
| Lys-Leu-Val-Phe-Phe-Ala | (SEQ ID NO:3); |
| Lys-Phe-Val-Phe-Phe-Ala | (SEQ ID NO:4); |
| Ala-Phe-Phe-Val-Leu-Lys | (SEQ ID NO:5); |
| Lys-Leu-Val-Phe | (SEQ ID NO:6); |
| Lys-Ala-Val-Phe-Phe-Ala | (SEQ ID NO:7); |
| Lys-Leu-Val-Phe-Phe | (SEQ ID NO:8); |
| Lys-Val-Val-Phe-Phe-Ala | (SEQ ID NO:9); |
| Lys-Ile-Val-Phe-Phe-Ala-$NH_2$ | (SEQ ID NO:10); |
| Lys-Leu-Val-Phe-Phe-Ala-$NH_2$ | (SEQ ID NO:11); |
| Lys-Phe-Val-Phe-Phe-Ala-$NH_2$ | (SEQ ID NO:12); |
| Ala-Phe-Phe-Val-Leu-Lys-$NH_2$ | (SEQ ID NO:13); |
| Lys-Leu-Val-Phe-$NH_2$ | (SEQ ID NO:14); |
| Lys-Ala-Val-Phe-Phe-Ala-$NH_2$ | (SEQ ID NO:15); |
| Lys-Leu-Val-Phe-Phe-$NH_2$ | (SEQ ID NO:16); |
| Lys-Val-Val-Phe-Phe-Ala-$NH_2$ | (SEQ ID NO:17); |
| Lys-Leu-Val-Phe-Phe-Ala-Gln | (SEQ ID NO:18); |
| Lys-Leu-Val-Phe-Phe-Ala-Gln-$NH_2$ | (SEQ ID NO:19); |
| His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-$NH_2$ | (SEQ ID NO:20); |
| His-His-Gln-Lys | (SEQ ID NO:23); and |
| Gln-Lys-Leu-Val-Phe-Phe-$NH_2$ | (SEQ ID NO:24). |

8. (amended once) The antifibrillogenic agent of claim 1, wherein the peptide of formula I is a peptide as set forth in SEQ ID NO:2.

15. (amended once) The labeled conjugate of claim 9, wherein said peptide of Formula I is selected from the group consisting of:

| | |
|---|---|
| Lys-Ile-Val-Phe-Phe-Ala | (SEQ ID NO:1); |
| Lys-Lys-Leu-Val-Phe-Phe-Ala | (SEQ ID NO:2); |
| Lys-Leu-Val-Phe-Phe-Ala | (SEQ ID NO:3); |
| Lys-Phe-Val-Phe-Phe-Ala | (SEQ ID NO:4); |
| Ala-Phe-Phe-Val-Leu-Lys | (SEQ ID NO:5); |
| Lys-Leu-Val-Phe | (SEQ ID NO:6); |
| Lys-Ala-Val-Phe-Phe-Ala | (SEQ ID NO:7); |
| Lys-Leu-Val-Phe-Phe | (SEQ ID NO:8); |
| Lys-Val-Val-Phe-Phe-Ala | (SEQ ID NO:9); |
| Lys-Ile-Val-Phe-Phe-Ala-$NH_2$ | (SEQ ID NO:10); |
| Lys-Leu-Val-Phe-Phe-Ala-$NH_2$ | (SEQ ID NO:11); |
| Lys-Phe-Val-Phe-Phe-Ala-$NH_2$ | (SEQ ID NO:12); |
| Ala-Phe-Phe-Val-Leu-Lys-$NH_2$ | (SEQ ID NO:13); |
| Lys-Leu-Val-Phe-$NH_2$ | (SEQ ID NO:14); |
| Lys-Ala-Val-Phe-Phe-Ala-$NH_2$ | (SEQ ID NO:15); |
| Lys-Leu-Val-Phe-Phe-$NH_2$ | (SEQ ID NO:16); |
| Lys-Val-Val-Phe-Phe-Ala-$NH_2$ | (SEQ ID NO:17); |
| Lys-Leu-Val-Phe-Phe-Ala-Gln | (SEQ ID NO:18); |
| Lys-Leu-Val-Phe-Phe-Ala-Gln-$NH_2$ | (SEQ ID NO:19); |
| His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-$NH_2$ | (SEQ ID NO:20); |
| His-His-Gln-Lys | (SEQ ID NO:23); and |
| Gln-Lys-Leu-Val-Phe-Phe-NH2 | (SEQ ID NO:24). |

18. (amended once) A method for the treatment of amyloidosis disorders in a patient, which comprises administering to said patient a therapeutically effective amount of a peptide of Formula I as defined in claim 1.

19. (amended once) A method for the treatment of amyloidosis disorders in a patient, which comprises administering to said patient a therapeutically effective amount of an antifibrillogenic agent as defined in claim 1.

20. (amended once) A composition for the treatment of amyloidosis disorders in a patient, which comprises a therapeutically effective amount of a peptide of Formula I as defined in claim 1 in association with a pharmaceutically acceptable carrier.

21. (amended once) A composition for the treatment of amyloidosis disorders in a patient, which comprises a therapeutically effective amount of an antifibrillogenic agent as defined in claim 1 in association with a pharmaceutically acceptable carrier.

22. (amended once) A composition for *in vivo* imaging of amyloid deposits, which comprises a therapeutically effective amount of a labeled conjugate as defined in claim 9 in association with a pharmaceutically acceptable carrier.

32. (amended once) A composition for inhibiting amyloidosis and/or for cytoprotection, which comprises a therapeutically effective amount of a peptide as defined in claim 31 in association with a pharmaceutically acceptable carrier.

34. (amended once) A process for the preparation of cells suitable for transplantation into a mammal, which cells are capable of forming amyloid deposits, said process comprising contacting the cells *in vitro* with the peptide of Formula I as defined in claim 1.

35. (amended once) The process of claim 34, wherein said peptide of Formula I or said antifibrillogenic compound causes breakdown of amyloid deposits, the deposits having been formed by said cells prior to said contact.

36. (amended once) The process of claim 34 , in which the cells

37. (new) The antifibrillogenic agent of claim 1, wherein the peptide of formula I is a peptide as set forth in SEQ ID NO:3.

38. (new) A process for the preparation of cells suitable for transplantation into a mammal, which cells are capable of forming amyloid deposits, said process comprising contacting the cells *in vitro* with the antifibrillogenic compound as defined in claim 1 for inhibiting amyloid deposit formation.

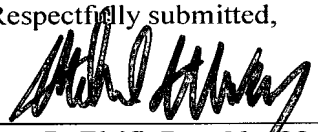
*Pursuant to 37 CFR 1.121(c)(1)(ii), a marked up version of the claims showing the changes made appears as Appendix A of this Amendment.*

## REMARKS

Applicant has amended the claims so as to clarify and more particularly indicate the claimed subject matter, and to remove multiple dependencies and informalities. The amendment is made for the sole purpose of expediting prosecution and not in response to any ground or reason of patentability presented by the USPTO. No new matter is added. Accordingly, claims 1-22, 29, 32, and 34-38 are pending in the present application.

On the basis of the foregoing amendments, Applicants respectfully submit that the pending claims are in condition for allowance. If there are any questions regarding these amendments and remarks, the Examiner is encouraged to contact either of the undersigned at the telephone number provided below.

Respectfully submitted,



Ivor R. Elrifi, Reg. No. 39,529
Michel Morency, Limited Recognition
Nicholas P. Triano III, Reg. No. 36,397
Attorneys for Applicant(s)
MINTZ, LEVIN, COHN, FERRIS,
GLOVSKY and POPEO, P.C.
One Financial Center
Boston, Massachusetts 02111
Tel: (617) 542-6000
Fax: (617) 542-2241

Dated: November 5, 2001

**Appendix A: marked up version of the claims showing the changes made**

1. (amended once) An antifibrillogenic agent for inhibiting amyloidosis and/or for cytoprotection, which comprises a peptide of Formula I, an isomer thereof, a retro or a retro-inverso isomer thereof or a peptidomimetic thereof:

$$Xaa_1\text{-}Xaa_2\text{-}Xaa_3\text{-}Xaa_4 \qquad I$$

wherein,

$Xaa_1$ is selected from the group consisting of Lys- and $Xaa_5$-Lys-;

$Xaa_5$ is selected from the group consisting of Lys, His-Gln-, His-His-Gln-, Val-His-His-Gln-, Glu-Val-His-His-Gln-, Asp-Asp-Asp-, and Gln-;

$Xaa_2$ is any amino acid;

$Xaa_3$ is Val;

$Xaa_4$ is selected from the group consisting of Phe, Phe-$NH_2$, Phe-Phe, Phe-Phe-Ala, Phe-Phe-Ala-$NH_2$, Phe-Phe-Ala-Gln, and Phe-Phe-Ala-Gln-$NH_2$;

wherein said peptide has at least one [D] amino acid residue,

with the proviso that Lys-Lys-Leu-Val-Phe-Phe-Ala is an all-[D] peptide.

2. The antifibrillogenic agent of claim 1, wherein $Xaa_2$ is a hydrophobic amino acid residue.

3. The antifibrillogenic agent of claim 1, wherein the peptide of formula I has at least two [D] amino acid residues.

4. The antifibrillogenic agent of claim 1, wherein the peptide of formula I has at least three [D] amino acid residues.

5. The antifibrillogenic agent of claim 1, wherein the peptide of formula I has one [L] amino acid residue.

6. The antifibrillogenic agent of claim 1, wherein the peptide of formula I is an all-[D] isomer peptide.

7. (amended once) The antifibrillogenic agent of claim 1~~, 2, 3, 4, 5, or 6~~, wherein said peptide of Formula I is selected from the group consisting of:

| | |
|---|---|
| Lys-Ile-Val-Phe-Phe-Ala | (SEQ ID NO:1); |
| Lys-Lys-Leu-Val-Phe-Phe-Ala | (SEQ ID NO:2); |
| Lys-Leu-Val-Phe-Phe-Ala | (SEQ ID NO:3); |
| Lys-Phe-Val-Phe-Phe-Ala | (SEQ ID NO:4); |
| Ala-Phe-Phe-Val-Leu-Lys | (SEQ ID NO:5); |
| Lys-Leu-Val-Phe | (SEQ ID NO:6); |
| Lys-Ala-Val-Phe-Phe-Ala | (SEQ ID NO:7); |
| Lys-Leu-Val-Phe-Phe | (SEQ ID NO:8); |
| Lys-Val-Val-Phe-Phe-Ala | (SEQ ID NO:9); |
| Lys-Ile-Val-Phe-Phe-Ala-$NH_2$ | (SEQ ID NO:10); |
| Lys-Leu-Val-Phe-Phe-Ala-$NH_2$ | (SEQ ID NO:11); |
| Lys-Phe-Val-Phe-Phe-Ala-$NH_2$ | (SEQ ID NO:12); |
| Ala-Phe-Phe-Val-Leu-Lys-$NH_2$ | (SEQ ID NO:13); |
| Lys-Leu-Val-Phe-$NH_2$ | (SEQ ID NO:14); |
| Lys-Ala-Val-Phe-Phe-Ala-$NH_2$ | (SEQ ID NO:15); |
| Lys-Leu-Val-Phe-Phe-$NH_2$ | (SEQ ID NO:16); |
| Lys-Val-Val-Phe-Phe-Ala-$NH_2$ | (SEQ ID NO:17); |
| Lys-Leu-Val-Phe-Phe-Ala-Gln | (SEQ ID NO:18); |
| Lys-Leu-Val-Phe-Phe-Ala-Gln-$NH_2$ | (SEQ ID NO:19); |
| His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-$NH_2$ | (SEQ ID NO:20); |
| His-His-Gln-Lys | (SEQ ID NO:23); and |
| Gln-Lys-Leu-Val-Phe-Phe-$NH_2$ | (SEQ ID NO:24). |

8. (amended once) The antifibrillogenic agent of claim 1, wherein the peptide of formula I is a peptide as set forth in SEQ ID NO:2~~ or SEQ ID NO:3~~.

9. A labeled conjugate for *in vivo* imaging of amyloid deposits, which comprises a conjugate of formula II:

A-B-C II

wherein A is an amyloid plaque-targeting moiety selected from the group consisting of a peptide of Formula I as defined in claim 1, an isomer thereof, a retro or a retro-inverso isomer thereof and a peptidomimetic thereof,

wherein B is a linker portion allowing attachment of the amyloid plaque-targeting moiety to C; and

wherein C is a label that allows for said *in vivo* imaging.

10. The labeled conjugate of claim 9, wherein $Xaa_2$ in Formula I is a hydrophobic amino acid residue.

11. The labeled conjugate of claim 9, wherein the peptide of formula I has at least two [D] amino acid residues.

12. The labeled conjugate of claim 9, wherein the peptide of formula I has at least three [D] amino acid residues.

13. The labeled conjugate of claim 9, wherein the peptide of formula I has one [L] amino acid residue.

14. The labeled conjugate of claim 9, wherein the peptide of formula I is an all-[D] isomer peptide.

15. (amended once) The labeled conjugate of claim ~~9, 10, 11, 12, 13 or 14~~, wherein said peptide of Formula I is selected from the group consisting of:

| | |
|---|---|
| Lys-Ile-Val-Phe-Phe-Ala | (SEQ ID NO:1); |
| Lys-Lys-Leu-Val-Phe-Phe-Ala | (SEQ ID NO:2); |
| Lys-Leu-Val-Phe-Phe-Ala | (SEQ ID NO:3); |
| Lys-Phe-Val-Phe-Phe-Ala | (SEQ ID NO:4); |
| Ala-Phe-Phe-Val-Leu-Lys | (SEQ ID NO:5); |
| Lys-Leu-Val-Phe | (SEQ ID NO:6); |
| Lys-Ala-Val-Phe-Phe-Ala | (SEQ ID NO:7); |

| | |
|---|---|
| Lys-Leu-Val-Phe-Phe | (SEQ ID NO:8); |
| Lys-Val-Val-Phe-Phe-Ala | (SEQ ID NO:9); |
| Lys-Ile-Val-Phe-Phe-Ala-$NH_2$ | (SEQ ID NO:10); |
| Lys-Leu-Val-Phe-Phe-Ala-$NH_2$ | (SEQ ID NO:11); |
| Lys-Phe-Val-Phe-Phe-Ala-$NH_2$ | (SEQ ID NO:12); |
| Ala-Phe-Phe-Val-Leu-Lys-$NH_2$ | (SEQ ID NO:13); |
| Lys-Leu-Val-Phe-$NH_2$ | (SEQ ID NO:14); |
| Lys-Ala-Val-Phe-Phe-Ala-$NH_2$ | (SEQ ID NO:15); |
| Lys-Leu-Val-Phe-Phe-$NH_2$ | (SEQ ID NO:16); |
| Lys-Val-Val-Phe-Phe-Ala-$NH_2$ | (SEQ ID NO:17); |
| Lys-Leu-Val-Phe-Phe-Ala-Gln | (SEQ ID NO:18); |
| Lys-Leu-Val-Phe-Phe-Ala-Gln-$NH_2$ | (SEQ ID NO:19); |
| His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-$NH_2$ | (SEQ ID NO:20); |
| His-His-Gln-Lys | (SEQ ID NO:23); and |
| Gln-Lys-Leu-Val-Phe-Phe-NH2 | (SEQ ID NO:24). |

16. The labeled conjugate of claim 15, wherein B is selected from the group consisting of Glucose and Phe.

17. The labeled conjugate of claim 15, wherein C is $^{99m}Tc$.

18. (amended once) A method for the treatment of amyloidosis disorders in a patient, which comprises administering to said patient a therapeutically effective amount of a peptide of Formula I as defined in claim 1~~, 2, 3, 4, 5, 6, 7 or 8~~.

19. (amended once) A method for the treatment of amyloidosis disorders in a patient, which comprises administering to said patient a therapeutically effective amount of an antifibrillogenic agent as defined in claim 1~~, 2, 3, 4, 5, 6, 7 or 8~~.

20. (amended once) A composition for the treatment of amyloidosis disorders in a patient, which comprises a therapeutically effective amount of a peptide of Formula I as defined in claim 1~~, 2, 3, 4, 5, 6, 7 or 8~~ in association with a pharmaceutically acceptable carrier.

21. (amended once) A composition for the treatment of amyloidosis disorders in a patient, which comprises a therapeutically effective amount of an antifibrillogenic agent as defined in claim 1~~, 2, 3, 4, 5, 6, 7 or 8~~ in association with a pharmaceutically acceptable carrier.

22. (amended once) A composition for *in vivo* imaging of amyloid deposits, which comprises a therapeutically effective amount of a labeled conjugate as defined in claim 9~~, 10, 11, 12, 13, 14, 15, 16 or 17~~ in association with a pharmaceutically acceptable carrier.

~~23. Use of a peptide of Formula I as defined in claim 1, 2, 3, 4, 5, 6, 7 or 8 for inhibiting amyloidosis and/or for cytoprotection.~~

~~24. Use of an antifibrillogenic agent as defined in claim 1, 2, 3, 4, 5, 6, 7 or 8 for inhibiting amyloidosis and/or for cytoprotection.~~

~~25. Use of a labeled conjugate as defined in claim 10, 11, 12, 13, 14, 15, 16 or 17 for *in vivo* imaging of amyloid deposits.~~

~~26. Use of a peptide of Formula I as defined in claim 1, 2, 3, 4, 5, 6, 7 or 8 for the manufacture of a medicament for inhibiting amyloidosis and/or for cytoprotection.~~

~~27. Use of an antifibrillogenic agent as defined in claim 1, 2, 3, 4, 5, 6, 7 or 8 for the manufacture of a medicament for inhibiting amyloidosis and/or for cytoprotection.~~

~~28. Use of a labeled conjugate as defined in claim 10, 11, 12, 13, 14, 15, 16 or 17 for the manufacture of a medicament for *in vivo* imaging of amyloid deposits.~~

29. A peptide, an isomer thereof, a retro or a retro-inverso isomer thereof or a peptidomimetic thereof, for use in inhibiting amyloidosis and/or for cytoprotection, said peptide having a sequence taken from the β-sheet region of an amyloid protein selected from the group consisting of IAPP and protease resistant prion protein.

~~30. Use of a peptide as defined in claim 29 for inhibiting amyloidosis and/or for cytoprotection.~~

~~31. Use of a peptide as defined in claim 29 for the manufacture of a medicament for inhibiting amyloidosis and/or for cytoprotection.~~

32. (amended once) A composition for inhibiting amyloidosis and/or for cytoprotection, which comprises a therapeutically effective amount of a peptide as defined in claim 31~~, 30 or 31~~ in association with a pharmaceutically acceptable carrier.

~~33. Use of a labeled peptide as defined in claim 29 for the manufacture of a medicament for *in vivo* imaging of amyloid deposits.~~

34. (amended once) A process for the preparation of cells suitable for transplantation into a mammal, which cells are capable of forming amyloid deposits, said process comprising contacting the cells *in vitro* with the peptide of Formula I as defined in claim 1 ~~or with the antifibrillogenic compound as defined in claim 1, 2, 3, 4, 5, 6, 7 or 8 for inhibiting amyloid deposit formation~~.

35. (amended once) ~~Process~~ The process ~~according to~~ of claim 34, wherein said peptide of Formula I or said antifibrillogenic compound causes breakdown of amyloid deposits, the deposits having been formed by said cells prior to said contact.

36. (amended once) The ~~P~~process ~~according to~~ of claim 34 ~~or 35~~, in which the cells are cultured in the presence of the peptide of Formula I or the antifibrillogenic compound.

37. (new) The antifibrillogenic agent of claim 1, wherein the peptide of formula I is a peptide as set forth in SEQ ID NO:3.

38. (new) A process for the preparation of cells suitable for transplantation into a mammal, which cells are capable of forming amyloid deposits, said process comprising contacting the cells *in vitro* with the antifibrillogenic compound as defined in claim 1 for inhibiting amyloid deposit formation.

10 Rec'd PCT/PTO 03 NOV 2001

# STEREOSELECTIVE ANTIFIBRILLOGENIC PEPTIDES AND PEPTIDOMIMETICS THEREOF

## BACKGROUND OF THE INVENTION

(a) Field of the Invention

The invention relates to agents having potent antifibrillogenic activity for the treatment of amyloidosis disorders and for imaging of amyloid deposits. These agents include peptides and peptidomimetic compounds thereof.

(b) Description of Prior Art

Amyloidosis refers to a pathological condition characterized by the presence of amyloid fibers. Amyloid is a generic term referring to a group of diverse but specific extracellular protein deposits that are seen in a number of different diseases. Though diverse in their occurrence, all amyloid deposits share common morphologic properties, stain with specific dyes (e.g. Congo red), and have a characteristic red-green birefringent appearance in polarized light after staining. They also share common ultrastructural, x-ray diffraction and infrared spectra features.

Some amyloidotic diseases can be idiopathic but most of these diseases appear as a complication of a previously existing disorder. For example, primary amyloidosis can appear without any other pathology or can follow plasma cell dyscrasia or multiple myeloma. Secondary amyloidosis is usually seen associated with chronic infection (such as tuberculosis) or chronic inflammation (such as rheumatoid arthritis). A familial form of secondary amyloidosis is also seen in Familial Mediterranean Fever (FMF). This familial type of amyloidosis, as one of the other types of familial amyloidosis, is genetically inherited and is found in specific population groups. Isolated forms of amyloidosis are those that tend to involve a single

organ system. Different amyloids are also characterized by the type of protein present in the deposit. For example, neurodegenerative diseases such as scrapie, bovine spongiform encephalitis, Creutzfeldt-Jakob disease and the like are characterized by the appearance and accumulation of a protease-resistant form of a prion protein (referred to as AScr or PrP-27) in the central nervous system. Similarly, Alzheimer's disease, another neurodegenerative disorder, is characterized by congophilic cerebral angiopathy, neuritic plaques and neurofibrillary tangles. In this case, the plaque and blood vessel amyloid is formed by the deposition of fibrillar Aβ amyloid protein. In adult-onset diabetes, amyloids containing the IAPP amyloid protein accumulate in the pancreas. Other systemic diseases, complications of long-term hemodialysis and sequelae of long-standing inflammation or plasma cell dyscrasias are characterized by the accumulation of amyloids systemically. In each of these cases, a different amyloidogenic protein is involved in amyloid deposition.

Once these amyloids have formed, there is no known, widely accepted therapy or treatment that significantly dissolves the deposits *in situ*.

Each amyloidogenic protein has the ability to organize into β-sheet and to form insoluble fibrils that get deposited extracellularly. Each amyloidogenic protein, although different in amino acid sequence has the same property of forming fibrils and binding to other elements such as proteoglycan (glycosaminoglycan), amyloid P and complement component. Moreover, each amyloidogenic protein has amino acid sequences which, although different, will show similarities such as regions with the ability to

ART 34 AMDT

bind to GAG's (referred to as the GAG binding site) as well as other regions which will promote β-sheet formation referred to as β-sheet region.

In specific cases, amyloidotic fibrils once deposited can become toxic to the surrounding cells. As per example, the Aβ fibrils organized as senile plaques have been shown to be associated with dead neuronal cells and microgliosis in patients with Alzheimer's disease. When tested *in vitro*, Aβ peptide was shown to be capable of triggering an activation process of the microglia (brain macrophages), which would explain the presence of microgliosis and brain inflammation found in the brain of patients with Alzheimer's disease.

In another type of amyloidosis seen in patients with Type II diabetes, the amyloidogenic protein IAPP, has been shown to induce β-islet cell toxicity *in vitro*. Hence, appearance of IAPP fibrils in the pancreas of Type II diabetic patients could contribute to the loss of the β islet cells (Langerhans) and organ dysfunction.

Particularly, in patients with Alzheimer's Disease, an agent capable 1) of preventing amyloid fibril formation and deposition and 2) of directly or indirectly inhibiting Aβ-induced neurotoxicity and inflammation (microgliosis), could be a treatment of choice to prevent and arrest the development of Alzheimer's disease.

WO-A-9808868 concerns compounds that modulate natural beta-amyloid peptide aggregation. The compounds comprise a peptide, preferably based on a beta -amyloid peptide, that is comprised of 3-5 D-amino acid residues and includes at least two D-amino acid residues independently selected from the group consisting of D-

ART 34 AMDT



leucine, D-phenylalanine and D-valine. In one embodiment the peptide is a retro-inverso isomer of a beta -amyloid peptide. In certain embodiments, the peptide is modified at the amino-terminus, the carboxy-terminus, or both.

It would be highly desirable to be provided with agents having potent antifibrillogenic activity for the treatment of amyloidosis disorders.

## SUMMARY OF THE INVENTION

One aim of the present invention is to provide agents having potent antifibrillogenic activity for the treatment of amyloidosis disorders.

Another aim of the present invention is to provide a method for the treatment of amyloidosis disorders, such as Alzheimer's' disease.

A number of strategies for possible therapeutic intervention in amyloid development have been proposed. These strategies include reduction of the pool of precursor proteins, prevention of the interaction of precursor proteins and disruption of preformed amyloid. The present invention deals mainly with the second approach, prevention of precursor protein interactions. The ideal molecule to fulfill this function, would interact specifically with the amyloid protein and would in so doing prevent the protein from interacting with itself. When dealing with molecules that are chiral, it is standard practice to identify which of the stereoisomers possesses the activity, since in general, activity can be attributed to one or the other of the isomers. By using a stereochemically pure isomer, side reactions can be avoided or reduced.

In accordance with one embodiment of the present invention there is provided an antifibrillogenic agent for inhibiting amyloidosis and/or for cytoprotection, which comprises a peptide of Formula I, an isomer thereof, a retro or a retro-inverso isomer thereof or a peptidomimetic thereof:

$$Xaa_1\text{-}Xaa_2\text{-}Xaa_3\text{-}Xaa_4 \qquad I$$

wherein,

$Xaa_1$ is absent or selected from the group consisting of Lys, Lys-Lys, $Xaa_5$-Lys-, and Ala;

$Xaa_5$ is absent or selected from the group consisting of His-Gln-, His-His-Gln-, Val-His-His-Gln-, Glu-Val-His-His-Gln-, Asp-Asp-Asp-, Lys-Val-Asp-Asp-Gln-Asp-, Gln-;
$Xaa_2$ is absent or any amino acid;
$Xaa_3$ is absent, Val or Phe;
$Xaa_4$ is absent or selected from the group consisting of Phe, Phe-$NH_2$, Phe-Phe, Phe-Phe-Ala, Phe-Phe-Ala-$NH_2$, Phe-Phe-Ala-Gln, Phe-Phe-Ala-Gln-$NH_2$, Val-Leu-Lys, Val-Leu-Lys-$NH_2$;
wherein the peptide of formula I contains at least one Lys or Asp;
and wherein the peptide has at least one [D] amino acid residue,
with the proviso that Lys-Lys-Leu-Val-Phe-Phe-Ala is an all-[D] peptide; and with the proviso that when $Xaa_5$ is Lys-Val-Asp-Asp-Gln-Asp- all of $Xaa_2$, $Xaa_3$, and $Xaa_4$ are absent.

Preferably, $Xaa_2$ is a hydrophobic amino acid residue such as a leucine residue.

In one embodiment of the invention, the peptide of formula I has at least two [D] amino acid residues, and more preferably at least three [D] amino acid residues. Optionally, the peptide of formula I has one [L] amino acid residue, or more preferably the peptide is an all-[D] isomer peptide.

In another embodiment of the invention, the peptide of Formula I is selected from the group consisting of:

| | |
|---|---|
| Lys-Ile-Val-Phe-Phe-Ala | (SEQ ID NO:1); |
| Lys-Lys-Leu-Val-Phe-Phe-Ala | (SEQ ID NO:2); |
| Lys-Leu-Val-Phe-Phe-Ala | (SEQ ID NO:3); |
| Lys-Phe-Val-Phe-Phe-Ala | (SEQ ID NO:4); |
| Ala-Phe-Phe-Val-Leu-Lys | (SEQ ID NO:5); |
| Lys-Leu-Val-Phe | (SEQ ID NO:6); |
| Lys-Ala-Val-Phe-Phe-Ala | (SEQ ID NO:7); |

Lys-Leu-Val-Phe-Phe (SEQ ID NO:8);
Lys-Val-Val-Phe-Phe-Ala (SEQ ID NO:9);
Lys-Ile-Val-Phe-Phe-Ala-$NH_2$ (SEQ ID NO:10);
Lys-Leu-Val-Phe-Phe-Ala-$NH_2$ (SEQ ID NO:11);
Lys-Phe-Val-Phe-Phe-Ala-$NH_2$ (SEQ ID NO:12);
Ala-Phe-Phe-Val-Leu-Lys-$NH_2$ (SEQ ID NO:13);
Lys-Leu-Val-Phe-$NH_2$ (SEQ ID NO:14);
Lys-Ala-Val-Phe-Phe-Ala-$NH_2$ (SEQ ID NO:15);
Lys-Leu-Val-Phe-Phe-$NH_2$ (SEQ ID NO:16);
Lys-Val-Val-Phe-Phe-Ala-$NH_2$ (SEQ ID NO:17);
Lys-Leu-Val-Phe-Phe-Ala-Gln (SEQ ID NO:18);
Lys-Leu-Val-Phe-Phe-Ala-Gln-$NH_2$ (SEQ ID NO:19);
His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-$NH_2$ (SEQ ID NO:20);
Asp-Asp-Asp (SEQ ID NO:21);
Lys-Val-Asp-Asp-Gln-Asp (SEQ ID NO:22);
His-His-Gln-Lys (SEQ ID NO:23);
and
Gln-Lys-Leu-Val-Phe-Phe-$NH_2$ (SEQ ID NO:24).

More preferably, the peptide of formula I is a peptide as set forth in SEQ ID NO:2 or SEQ ID NO:3.

In accordance with one embodiment of the present invention there is provided a labeled conjugate for *in vivo* imaging of amyloid plaque, which comprises a conjugate of formula II:

A-B-C II

wherein A is an amyloid plaque-targeting moiety selected from the group consisting of a peptide of Formula I as defined above, an isomer thereof, a retro or a retro-inverso isomer thereof and a peptidomimetic thereof,
wherein B is a linker portion allowing attachment of the amyloid plaque-targeting moiety to C; and
wherein C is a label that allows for *in vivo* imaging.

Preferably, the linker portion B is selected from the group consisting of Glucose and Phe. Preferably, the label C is $^{99m}Tc$.

Still in accordance with the present invention, there is provided a method for the treatment of amyloidosis disorders in a patient, which comprises administering to the patient a therapeutically effective amount of a peptide of Formula I, or the antifibrillogenic agent, as defined above.

Further in accordance with the present invention, there is provided a composition for the treatment of amyloidosis disorders in a patient, which comprises a therapeutically effective amount of a peptide of Formula I, or of an antifibrillogenic agent, as defined above in association with a pharmaceutically acceptable carrier.

In accordance with the present invention, there is also provided a composition for *in vivo* imaging of amyloid plaques, which comprises a therapeutically effective amount of a labeled conjugate as defined above in association with a pharmaceutically acceptable carrier.

The peptide of Formula I or the antifibrillogenic agent may be used for inhibiting amyloidosis and/or for cytoprotection.

The labeled conjugate may be used for *in vivo* imaging of amyloid plaques.

The peptide of Formula I or the antifibrillogenic agent may alternatively be used for the manufacture of a medicament for inhibiting amyloidosis and/or for cytoprotection.

Similarly, the labeled conjugate may also be used for the manufacture of a medicament for *in vivo* imaging of amyloid plaques.

Other embodiments of these peptides include racemic mixtures and peptides having mixed chirality, i.e., different chirality at different chiral centers.

In accordance with the peptides Lys-Lys-Leu-Val-Phe-Phe-Ala (SEQ ID NO:2) and Lys-Leu-Val-Phe-Phe-Ala (SEQ ID NO:3), one stereoisomer, the D form, is found to be more active than the L form, and the D isomer is the preferred form for use of this peptide as a drug.

The present invention further provides similar peptides designed for the other amyloidogenic peptides such as AA, AL, and IAPP. In fact, the present invention also provides a peptide for inhibiting amyloidosis and/or for cytoprotection. The peptide has a sequence taken from the β-sheet region of an amyloid protein. Such peptide or a composition containing such peptide can be used for inhibiting amyloidosis and/or for cytoprotection. Alternatively, such peptide or a composition containing such peptide can be used for the manufacture of a medicament for inhibiting amyloidosis and/or for cytoprotection.

Accordingly, the present invention also provides a composition for inhibiting amyloidosis and/or for cytoprotection, which comprises a therapeutically effective amount of a peptide as defined previously in association with a pharmaceutically acceptable carrier.

In accordance with the present invention, the amyloidosis disorder includes, without limitation, prion protein related disorders, type II diabetes and Alzheimer's disease.

With regard to another aspect of the invention, diseases caused by the death or malfunctioning of a particular type or types of cells can be treated by transplanting into the patient

healthy cells of the relevant type of cell. Often these cells are cultured *in vitro* prior to transplantation to increase their numbers, to allow them to recover after the isolation procedure or to reduce their immunogenicity. However, in many instances the transplants are unsuccessful, due to the death of the transplanted cells. The inventors have now also found that culturing of cells can lead to the formation of fibrils from endogenous proteins. Such fibrils are likely to continue to grow after the cells are transplanted and cause death or dysfunction of the cells. The inventors have also found that the peptide of the present invention or the antifibrillogenic compound of the present invention can be used to reduce the formation of fibrils.

Thus the invention also provides a process for the preparation of cells suitable for transplantation into a mammal, which cells are capable of forming fibrils. The process comprises contacting the cells with the peptide of the present invention or the antifibrillogenic compound of the present invention.

The peptide of Formula I or the antifibrillogenic compound causes breakdown of amyloid deposits which have been formed by the cells prior to the contact. Preferably, the cells are cultured in the presence of the peptide of Formula I or the antifibrillogenic compound.

For the purpose of the present invention the following expressions and terms are defined below.

The term "agents having stereoselective antifibrillogenic activity" is intended to mean any peptides, peptide analogues, peptide derivatives, or peptidomimetics which retain the stereoselective antifibrillogenic activity, the cytoprotective and anti-inflammatory activity and/or the ability to alter

a natural amyloidotic protein aggregation as described herein. Peptide analogues, peptide derivatives, or peptidomimetics include any molecules that mimic the chemical structure of a peptide and retain the functional properties of the peptide (Williams, W.V. and Weiner, D.B., eds., Biologically Active Peptides: Design, Synthesis, and Utilization, vol. 1, Technomic Publishing Company Inc., Lancaster, Pa., 1993, pages 35-3..). Examples of peptide analogues, peptide derivatives, or peptidomimetics include compounds with sulfonamide, phosphoramide or non-amide linkages.

The expression "antifibrillogenic activity" is intended to mean the ability to block or prevent an amyloidogenic protein from forming fibrils, preferably by preventing it from adopting its β-pleated conformation.

The term "cytoprotection" or "cytoprotective activity" is intended to mean the ability to protect cells from amyloid-induced toxicity.

The expression "anti-inflammatory" is intended to mean the ability to block or reduce the Aβ-induced microglial activation process or to block the chemokine-induced inflammatory reaction.

The expression "retro isomer" is intended to mean a reversal of the direction of the peptide backbone.

The expression "inverso isomer" is intended to mean an inversion of the amino acid chirality used to make the peptide.

The expression "retro-inverso isomer" is intended to mean a reversal of both the peptide backbone direction and the amino acid chirality.

Except as otherwise expressly defined herein, the abbreviations used herein for designating the amino acids and the protective groups are based on

recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature (*Biochemistry*, 1972, **11**:1726-1732).

Also, unless specified otherwise, the Aβ(1-40) is the naturally occurring Aβ(1-40), that is the all [L]-isomer.

## BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates the targeted sites of the protein-protein interactions required for self-assembly into β-sheet fibrils;

Fig. 2 illustrates a thioflavin T fluorescence assay for fibril formation by [L]-Aβ (1-40) in the absence and presence of a peptide in accordance with one embodiment of the invention;

Fig. 3 shows the same assay as in Fig. 2 for fibril formation by [D]-Aβ (1-40);

Fig. 4 is a bar graph illustrating the percentage of thioflavin T fluorescence in the presence of the [D]-peptide used in Fig. 2, with or without single substitutions of corresponding [L]-amino acids;

Fig. 5 is a bar graph illustrating a thioflavin T fluorescence assay for fibril formation by [L]-Aβ (1-40) in the presence of the [D]-peptide used in Fig. 2, with or without substitution of the Leu residue by other hydrophobic amino acids;

Fig. 6 illustrates the toxicity of [L]-Aβ (1-40) in the absence and presence of peptides in accordance with one embodiment of the invention; and

Fig. 7 is a bar graph illustrating the toxicity of [L]-Aβ (1-40) in the presence of another peptide of the present invention.

## DETAILED DESCRIPTION OF THE INVENTION

As illustrated in Fig. 1, internal regions of the Aβ sequence have been shown to confer characteristics of the amyloid protein. Indeed, the region between amino acid 13-16 (His-His-Gln-Lys, SEQ ID NO:23) of the amyloid protein is responsible for the interaction between the Aβ protein and the glycosaminoglycan moiety of the proteoglycans (Kisilevsky, R., et al., Proteoglycans and amyloid fibrillogenesis: The nature and origin of amyloid fibrils, Wiley, Chichester (*CIBA Foundation Symposium 1997*), pp. 58-72). Proteoglycans are known to promote amyloid fibril formation as well as protect these fibrils from proteolysis (Gupta-Bansal, R., et al., 1995, *The Journal of Biological Chemistry*, **270**:18666-18671). More recently, the same region has been determined to play a role in the activation process of microglial cells by Aβ (Giulian, D., et al., 1998, *The Journal of Biological Chemistry*, **273**(45):29719-29726). This 13-16 region of Aβ, often referred to as the GAG binding site, is also part of a larger domain, the 10-16 region of the protein which has been suggested as the region responsible for the adherence of Aβ to the cell surface (Giulian, D., et al., 1996, *The Journal of Neuroscience*, **16**(19):6021-6037). Such adherence of Aβ to the cell surface will allow the interaction of Aβ with the specific cells leading to either microglia activation or toxicity of neuronal cells.

These two overlapping regions of the Aβ protein, i.e. amino acids 13-16 and 10-16 are adjacent to the 16-21 region of Aβ, a short hydrophobic stretch critical for the formation of fibrillar structures (Hilbrich, C., et al., 1992, *J. Mol. Biol.*, **228**:460-473). By having peptides capable of interacting with these overlapping regions of Aβ, one can aim at

preventing both Aβ fibril formation and Aβ cellular interaction (i.e. microglia activation, neurotoxicity).

A preferred embodiment of the present invention is novel and arises from the unexpected finding that the all-[D] stereoisomer peptides, Lys-Lys-Leu-Val-Phe-Phe-Ala (SEQ ID NO:2) and Lys-Leu-Val-Phe-Phe-Ala (SEQ ID NO:3), are much more potent inhibitors of Aβ(1-40) fibrillogenesis then the corresponding all-[L] peptides. The all-[D] stereoisomer peptides, Lys-Lys-Leu-Val-Phe-Phe-Ala (SEQ ID NO:2) and Lys-Leu-Val-Phe-Phe-Ala (SEQ ID NO:3) are also potent cytoprotective agents.

This finding was unforeseen particularly because the researchers who originally reported peptides containing the sequence Lys-Leu-Val-Phe-Phe-Ala (SEQ ID NO:3) as an inhibitor of fibrillogenesis, state in a second article which they published: "A peptide entirely composed of amino acids in D configuration with the sequence klvff (lowercase marks amino acids in D configuration) was synthesized using the SPOT technique and assayed for $^{125}$I-LBMP1620 binding. This peptide failed to bind $^{125}$I-LBMP1620 indicating that KLVFF-KLVFF interaction is sterospecific." Tjernberg, L.O. et al. (1997) Controlling Amyloid β-Peptide Fibril Formation with Protease-stable Ligands, *J. Biol. Chem.*, **272**:12602.

Inhibition of Amyloidosis

The experimental work performed leading to this invention included comparing the ability of the [D] and [L] stereoisomers of peptide Lys-Lys-Leu-Val-Phe-Phe-Ala (SEQ ID NO:2) to inhibit the fibrillogenesis process observed with the amyloidogenic peptide Aβ(1-40) in a thioflavin T fluorescence assay.

The thioflavin T fluorescence assay for fibrillogenesis is based on the principle that the fluorescent dye, thioflavin T, binds specifically to fibrillar, but not to unaggregated Aβ peptide (LeVine III, H., 1993, *Protein Science* **2**:404-410). Upon binding, thioflavin T develops a characteristic fluorescence (Naiki, H., et al., 1996, *Lab. Invest.* **74**: 374-383) which can be easily detected. The dye is believed to interact with the stacked cross-β pleated sheets, the common structural motif of all amyloids (LeVine III, H., 1995, *Amyloid: Int. J. Exp. Clin Invest.* **2**:1.6). Thioflavin T is widely used to assay the effect of compounds on Aβ peptide fibrillogenesis (Bronfman, P.C., et al., 1995, *Neuroscience Lett.* **218**:201-203).

In this assay test compounds are incubated with a solution of Aβ(1-40) (20 μM) containing 10 μM thioflavin T, in 0.02M Tris/0.02M acetate/0.15M NaCl/0.005% azide/pH 7.40 at 37°C in sealed 384 well microplates. Readings (ex 430 nm/em 485nm) are taken at various time intervals with a microplate fluorescence reader. An increase in fluorescence signifies the appearance of amyloid or intermediates in the production of amyloid. Inhibitors of fibrillogenesis will lead to less fluorescence production.

The results illustrated in Table 1 below, are based on the fluorescence production in the presence of test peptides at either 20 μM or 80 μM concentration, at the time intervals of 5, 19, 45, 67, 77 and 90 hours, compared to a control, buffer alone, without added inhibitory peptide.

Table 1

Order Of Potency of Peptide Inhibitors

| | Tested at 20μM | Tested at 80μM |
|---|---|---|
| (strongest activity) | 1 (D) KIVFFA | 1 (D) AFFVLK |
| | 2 (D) KKLVFFA | 1 (D) KKLVFFA |
| | 3 (D) KLVFFA | 1 (D) KLVFFA |
| | 4 (D) KFVFFA | 1 (D) KFVFFA |
| | 5 (D) AFFVLK | 5 (D) KIVFFA |
| | 6 (D) KLVF | 6 (D) KAVFFA |
| | 7 (D) KAVFFA | 7 (L) KKLVFFA |
| | 8 (L) KLVFFA | 8 (L) KLVFFA |
| | 9 (D) KLVFF | 9 (D) KLVF |
| | 10 (L) KKLVFFA | 10 (D) KLVFF |
| (weakest activity) | 11 (L) AFFVLK | 11 (L) AFFVLK |

Protocol

Aβ peptide: Aβ(1-40) 95% purity (American Peptide Company, Inc, Sunnyvale, Cal. USA, cat. 62-0-78) is disaggregated in trifluoroacetic acid and filtered through a 0.02 μM filter, (Whatman Anotop 25 plus, .02 μm, Catalogue no. 6809 4102) in hexafluoroisopropanol (HFIP). Solutions of Aβ(1-40) at 600 μM in HFIP are stored at -80°C.

Assay mixture: The mixture is prepared as two solutions that are combined upon addition to the 384 well microplate (Corning Costar cat. 3705).

i) Solution A consists of test peptides in 0.02M Tris/0.02M acetate/0.15M NaCl/0.01 % azide at pH 7.40 or buffer alone (control),

ii) Solution B consists of Aβ(1-40) 40 μM, thioflavin T 20 μM in 0.02M Tris/0.02M acetate/0.15M NaCl at pH 7.40. This solution is prepared by drying the Aβ peptide under

nitrogen and then resuspending this in 0.04M Tris base with 15 minutes sonication. An equal volume of 0.04M acetic acid containing 0.3 M NaCl is added and the solution is adjusted to pH 7.40±0.02. A small volume of 5mM thioflavin T is added to the solution to give a final 20 μM concentration of thioflavin T.

iii) The microplate is loaded with 40 μL of solution A followed by 40 μL of solution B which gives a final 20 μM Aβ(1 -40), 10 μM thioflavin T, and either 20 μM, 80 μM or 100 μM test compound in 0.02M Tris/0.02M acetate/0.15M NaCl/0.005% azide, pH 7.40. The plate is sealed and loaded into the microplate fluorescence reader.

Fluorescence measurement data analysis: The HTS-7000 Bio Assay Reader, Perkin Elmer, is used to perform kinetic runs of about 5 days. Readings were taken at various time intervals, 5, 19, 45, 67, 77 and 90 hours, with one minute shaking before each reading. Bandpass filters used were: excitation 430 nm, emission 485 mm.

**Calculations**

The rank order of efficacy of the peptides is determined by observing which peptides allow the appearance of fluorescence, above the background level, first. For example in the presence of buffer control alone, fluorescence appears earlier than when any of the peptides is present. The most active peptides prevent the appearance of fluorescence even after 90 hours of incubation.

The results achieved in the thioflavin T fibrillogenesis assays show that all-[D] stereoisomer peptide was about 60 times more potent then the all-[L] stereoisomer peptide. This is based on the observation that 400 μM all-[L] stereoisomer was required to give

an equivalent inhibition to that produced with 6.1 μM all-[D] stereoisomer peptide.

The results achieved in the Aβ-NBD environmental probe fibrillogenesis assay showed that the all-[D] stereoisomer peptide was at least 30 times more potent than the all-[L] stereoisomer peptide. This estimate is based on the observation that the lowest concentration of all-[D] peptide tested (25 μM) was more potent than the highest concentration of the all-[L] peptide (800 μM).

**β-sheet and GAG binding domains peptides**

Novel peptides and peptidomimetics that include complementary sequences to certain portions of amyloidogenic peptides such as Aβ, AA, AL, IAPP, and prion proteins are designed to be capable of inhibition of Protein-Protein interactions or self assembly. The targeted portions in the various disease-causing proteins aforementioned, preferably contain one or more charged residues such as aspartate, glutamate, lysine, histidine and arginine. Such peptides and their peptidomimetics will inhibit fibrillogenesis of the amyloidogenic peptides and prion proteins and interfere with chemokines binding to the cell surface proteoglycans leading to dimerization or tetramerization by interacting with their GAG binding domains. In the case of Aβ, these interactions lead to cytoprotection as well as inhibition of inflammatory response and serve as potent therapeutics for the treatment of Alzheimer's disease. In the case of chemokine-related disorders these interactions may lead to a decrease in the uncontrolled inflammatory response associated with some diseases.

Other amyloidogenic peptides such as IAPP, have also been tested. For example, 2 peptides from the β-sheet region of IAPP have been shown to inhibit IAPP

fibril formation using the thioflavin T fluorescence assay, circular dichroism (measures secondary structure) and the electron microscope (to look at fibrils directly).

The full-length IAPP is 37 amino acids and the β-sheet region is the 20-29 sequence. The 20-29 sequence is critical for forming β-sheet and has been previously shown to be a key region in modulating IAPP aggregation and folding. Hexapeptides from this β-sheet region were examined and 2 were found to be active.

Hexapeptides spanning the 20-29 region (Ser-Asn-Asn-Phe-Gly-Ala-Ile-Leu-Ser-Ser) of the IAPP protein were synthesized and tested for their ability to prevent fibril formation as determined by circular dichroism and the thioflavin T assay. Hexapeptides were designed and were found to be capable of blocking the formation of IAPP fibrils. These peptides (Ser-Asn-Asn-Phe-Gly-Ala- and Asn-Asn-Phe-Gly-Ala-Ile) were directed towards the central core of the 20-29 region.

Novel peptides containing 3-6 residues that are complementary (in terms of their charges) to the 10-16 segment of Aβ peptide have been shown for the first time to strongly interact with Aβ peptide. They provide a starting point for the design of BBB (blood brain barrier) permeable peptidomimetics. In principle, the present invention provides similar peptides can be designed for the other amyloidogenic peptides such as AA, AL, and IAPP.

Asp-Asp-Asp (SEQ ID NO:21), a tripeptide, when incubated with Aβ40 under physiological conditions shows a slight decrease at time t=0 in the amount of β-sheet content as is evident by the CD spectrum. Incubation of this tripeptide with Aβ40 for 24 hours shows no trace of β-sheet conformation of the Aβ40 and

clearly indicates the ability of this tripeptide to strongly interact with Aβ40 peptide and keep Aβ40 in a randomized and non-fibrillary conformation. The anti-fibrillogenic property of this tripeptide is also supported by the Aβ42 solubilization assay.

Lys-Val-Asp-Asp-Gln-Asp (SEQ ID NO:22), a hexapeptide, when incubated with Aβ40 under physiological conditions shows an increase at time t=0 in the amount of β-sheet content as is evident by the CD spectrum. Incubation of this hexapeptide with Aβ40 for 24 hours shows a dramatic increase in β-sheet content of the Aβ40 and clearly indicates the ability of this hexapeptide to strongly interact with Aβ40 peptide and organize it into a β-sheet conformation. Electron microscopy of the mixture failed to show any fibrils indicating that this particular compound is in fact an anti-fibrillogenic compound with regard to Aβ. *In vitro* results with NBD and thioflavin-T based fluorescence assays confirm this finding. It is the understanding of the inventors that this interesting observation will lead to a greater understanding of fibrillogenesis of Aβ40 and Aβ42 peptides and as a result, will provide important information for the design of potent anti-fibrillogenic compounds for Aβ, other amyloidotic peptides such as AA, AL and IAPP for the treatment of diseases such as Alzheimer's, Type II Diabetes and amyloidosis related disorders. The same principle can also be applied to the design of peptide type compounds for the inhibition of binding of various chemokines to the cell surface as well as inhibition of self assembly and cellular adherence of prion proteins.

The results illustrated in Fig. 2 show that all [D]-Lys-Leu-Val-Phe-Phe-Ala (SEQ. ID NO: 3) is a more potent inhibitor of Aβ (1-40) assembly in the thioflavin T fluorescence assay than is all [L]-Lys-

Leu-Val-Phe-Phe-Ala. Since the naturally occurring Aβ (1-40) used in these experiments was the all-[L] amino acid version, these results indicate that an inhibitor peptide works best when containing amino acids of the opposite chirality.

Fig. 3 demonstrates that the same rule of opposite chirality illustrated in Fig. 2 applies for the assembly of Aβ (1-40) synthesized using amino acids of the [D] type. In this experiment all-[L]-Lys-Leu-Val-Phe-Phe-Ala (SEQ. ID NO:3) is a more potent inhibitor in the all-[D]-Aβ (1-40) assembly reaction than all-[D]-Lys-Leu-Val-Phe-Phe-Ala. This result confirms that peptides of opposite chirality are better inhibitors.

Fig. 4 illustrates the inhibition of Aβ (1-40) fibril formation by all-[D]-Lys-Leu-Val-Phe-Phe-Ala (20μM) with or without single substitutions of [L]-amino acids in the thioflavin T fluorescence assay. In this experiment the ability of the all-[D]-Lys-Leu-Val-Phe-Phe-Ala peptide to inhibit Aβ (1-40) fibril formation, measured as percentage of thioflavin T fluorescence in the absence of peptide (control), was compared to [D]-Lys-Leu-Val-Phe-Phe-Ala peptides with single [L]-amino acid replacements. None of the mixed chirality Lys-Leu-Val-Phe-Phe-Ala peptides were more potent than the original all-[D] peptide. This result demonstrates that [D]-amino acids are more potent inhibitors of Aβ (1-40) fibrillogenesis than [L]-amino acids.

However as seen in Fig. 4 some peptides with single [L] substitutions do retain inhibitory activity. In particular peptides in which the [D] isomer of the Lys, the second Phe and the Ala are substituted with the [L]-isomers retain inhibitory activity. The substitutions, which reduce inhibitory activity the

most, are the Leu, the Val and the first Phe, indicating that these residues contribute the most to the potency of the [D]-peptide. From Fig. 4, it is apparent that peptides with mixed chirality or with at least one [D]-substituted amino acid are also inhibitors, although not as potent as the all-[D] peptide. These mixed-chirality peptides are thus meant to be included in the present invention.

Fig. 5 illustrates the inhibition of Aβ (1-40) fibril formation in the thioflavin T fluorescence assay by all-[D]-Lys-Leu-Val-Phe-Phe-Ala (20μM), with or without replacement of the leucine by other hydrophobic amino acids. In this experiment all the peptides tested retained some inhibitory activity. This result demonstrates that the leucine residue is not critical for inhibition of Aβ fibril formation in the all-[D] peptide. These results illustrated in Fig. 5 were non-obvious and unexpected in light of a prior publication which identified the Leucine residue as critical in an all-[L] version of the peptide (Tjernberg LO et al., *J. Biol. Chem.* **271**:8545, 1996).

**Cytoprotection**

The experimental work performed leading to this invention also included comparing the ability of [D] and [L] stereoisomers of the peptides of the present invention to show cytoprotective activity, i.e. to protect cells from Aβ toxicity.

Figure 6 uses the MTT assay on SH-SY5Y cells.

**Protocol**

A SH-SY5Y human neuroblast cell line (American Type Culture Collection, cat. CRL-2266) is cultured according to technical specifications. Monomerized Aβ (1-40) is prepared using trifluoroacetic acid and hexafluoroisopropanol, in the same way already described for the thioflavin T fluorescence assay.

Monomerized Aβ at various concentrations in TANA buffer (0.02 M TRIS base pH 7.4, 0.02M acetate, 0.15 M NaCl) is added to 100 μM test peptide and the mixture is incubated for 24 hours at 37°C with agitation, in order to allow polymerization to occur. Cells are adhered to a 96-well microplate for 2 hours at 37°C and 5% $CO_2$ before the Aβ-peptide mixture, or buffer alone (control), is added. The microplate is gently agitated and incubated for 20-24 hours at 37°C and 5% $CO_2$. Cell viability is determined by a MTT-based colorimetric assay. The MTT assay (Boehringer Mannheim, Cell Proliferation Kit 1) is based on the principle that the yellow tetrazolium salt MTT is cleaved in metabolically-active cells to produce purple formazan crystals. The formazan crystals are solubilized and the resulting colored solution is quantified using a scanning multiwell spectrophotometer (ELISA reader, Absorbance $A_{560}$ nm). Cellular toxicity was calculated as follows:

$$\text{Toxicity (\%)} = 100 - \frac{\text{(O.D. sample - O.D. Blank)}}{\text{(O.D. Control - O.D. Blank)}}.$$

Figure 6 shows the neurotoxicity of Aβ (1-40) in the absence or presence of various peptides of the present invention. In this experiment the all-[D]-Lys-Lys-Leu-Val-Phe-Phe-Ala (SEQ. ID NO: 2) peptide is a more potent inhibitor of Aβ neurotoxicity than the all-[L]-Lys-Lys-Leu-Val-Phe-Phe-Ala peptide in the cytoprotection assay.

Figure 7 uses the propidium iodide assay on primary cortical neurons. Briefly, fetal rat primary cortical neurons are isolated and cultured according to Durkin, J.P. et al., J. Neurochem., 66:951-962, 1996. Neurons are plated in a 48 well microplate. 7 days after plating the neuronal culture media is supplemented with B27 (Life Technologies, Data sheet

form No. 3755). A mixture of Aβ and test peptide is added to the cortical neurons for 3 days at 37°C and 5% CO2.

Cell viability is then visually assessed as the proportion of phase-bright cells that exclude propidium iodide, since only dead cells take up propidium iodide.

Figure 7 shows the potent cytoprotective activity of all-[D]-Lys-Leu-Val-Phe-Phe-Ala (SEQ ID NO:3). This experiment shows the potent cytoprotective activity of all-[D]-Lys-Leu-Val-Phe-Phe-Ala compared to Congo red, which is a known cytoprotective agent and compared to the absence of any cytoprotective agent (Aβ alone).

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth, and as follows in the scope of the appended claims.

ART 34 AMDT

**WHAT IS CLAIMED IS:**

1. An antifibrillogenic agent for inhibiting amyloidosis and/or for cytoprotection, which comprises a peptide of Formula I, an isomer thereof, a retro or a retro-inverso isomer thereof or a peptidomimetic thereof:

$Xaa_1$-$Xaa_2$-$Xaa_3$-$Xaa_4$ I

wherein,

$Xaa_1$ selected from the group consisting of Lys, $Xaa_5$-Lys-;

$Xaa_5$ is selected from the group consisting of Lys, His-Gln-, His-His-Gln-, Val-His-His-Gln-, Glu-Val-His-His-Gln-, Asp-Asp-Asp-, Gln-;

$Xaa_2$ is any amino acid;

$Xaa_3$ is Val;

$Xaa_4$ is selected from the group consisting of Phe, Phe-$NH_2$, Phe-Phe, Phe-Phe-Ala, Phe-Phe-Ala-$NH_2$, Phe-Phe-Ala-Gln, Phe-Phe-Ala-Gln-$NH_2$;

wherein said peptide has at least one [D] amino acid residue,

with the proviso that Lys-Lys-Leu-Val-Phe-Phe-Ala is an all-[D] peptide.

2. The antifibrillogenic agent of claim 1, wherein $Xaa_2$ is a hydrophobic amino acid residue.

3. The antifibrillogenic agent of claim 1, wherein the peptide of formula I has at least two [D] amino acid residues.

4. The antifibrillogenic agent of claim 1, wherein the peptide of formula I has at least three [D] amino acid residues.

ART 34 AMDT

5. The antifibrillogenic agent of claim 1, wherein the peptide of formula I has one [L] amino acid residue.

6. The antifibrillogenic agent of claim 1, wherein the peptide of formula I is an all-[D] isomer peptide.

7. The antifibrillogenic agent of claim 1, 2, 3, 4, 5, or 6, wherein said peptide of Formula I is selected from the group consisting of:

Lys-Ile-Val-Phe-Phe-Ala (SEQ ID NO:1);
Lys-Lys-Leu-Val-Phe-Phe-Ala (SEQ ID NO:2);
Lys-Leu-Val-Phe-Phe-Ala (SEQ ID NO:3);
Lys-Phe-Val-Phe-Phe-Ala (SEQ ID NO:4);
Ala-Phe-Phe-Val-Leu-Lys (SEQ ID NO:5);
Lys-Leu-Val-Phe (SEQ ID NO:6);
Lys-Ala-Val-Phe-Phe-Ala (SEQ ID NO:7);
Lys-Leu-Val-Phe-Phe (SEQ ID NO:8);
Lys-Val-Val-Phe-Phe-Ala (SEQ ID NO:9);
Lys-Ile-Val-Phe-Phe-Ala-$NH_2$ (SEQ ID NO:10);
Lys-Leu-Val-Phe-Phe-Ala-$NH_2$ (SEQ ID NO:11);
Lys-Phe-Val-Phe-Phe-Ala-$NH_2$ (SEQ ID NO:12);
Ala-Phe-Phe-Val-Leu-Lys-$NH_2$ (SEQ ID NO:13);
Lys-Leu-Val-Phe-$NH_2$ (SEQ ID NO:14);
Lys-Ala-Val-Phe-Phe-Ala-$NH_2$ (SEQ ID NO:15);
Lys-Leu-Val-Phe-Phe-$NH_2$ (SEQ ID NO:16);
Lys-Val-Val-Phe-Phe-Ala-$NH_2$ (SEQ ID NO:17);
Lys-Leu-Val-Phe-Phe-Ala-Gln (SEQ ID NO:18);
Lys-Leu-Val-Phe-Phe-Ala-Gln-$NH_2$ (SEQ ID NO:19);
His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-$NH_2$ (SEQ ID NO:20);
His-His-Gln-Lys (SEQ ID NO:23);
and
Gln-Lys-Leu-Val-Phe-Phe-$NH_2$ (SEQ ID NO:24).

ART 34 AMDT

8. The antifibrillogenic agent of claim 1, wherein the peptide of formula I is a peptide as set forth in SEQ ID NO:2 or SEQ ID NO:3.

9. A labeled conjugate for *in vivo* imaging of amyloid deposits, which comprises a conjugate of formula II:

A-B-C II

wherein A is an amyloid plaque-targeting moiety selected from the group consisting of a peptide of Formula I as defined in claim 1, an isomer thereof, a retro or a retro-inverso isomer thereof and a peptidomimetic thereof,
wherein B is a linker portion allowing attachment of the amyloid plaque-targeting moiety to C; and
wherein C is a label that allows for said *in vivo* imaging.

10. The labeled conjugate of claim 9, wherein $Xaa_2$ in Formula I is a hydrophobic amino acid residue.

11. The labeled conjugate of claim 9, wherein the peptide of formula I has at least two [D] amino acid residues.

12. The labeled conjugate of claim 9, wherein the peptide of formula I has at least three [D] amino acid residues.

13. The labeled conjugate of claim 9, wherein the peptide of formula I has one [L] amino acid residue.

14. The labeled conjugate of claim 9, wherein the peptide of formula I is an all-[D] isomer peptide.

ART 34 AMDT

15. The labeled conjugate of claim 9, 10, 11, 12, 13 or 14, wherein said peptide of Formula I is selected from the group consisting of:

Lys-Ile-Val-Phe-Phe-Ala (SEQ ID NO:1);
Lys-Lys-Leu-Val-Phe-Phe-Ala (SEQ ID NO:2);
Lys-Leu-Val-Phe-Phe-Ala (SEQ ID NO:3);
Lys-Phe-Val-Phe-Phe-Ala (SEQ ID NO:4);
Ala-Phe-Phe-Val-Leu-Lys (SEQ ID NO:5);
Lys-Leu-Val-Phe (SEQ ID NO:6);
Lys-Ala-Val-Phe-Phe-Ala (SEQ ID NO:7);
Lys-Leu-Val-Phe-Phe (SEQ ID NO:8);
Lys-Val-Val-Phe-Phe-Ala (SEQ ID NO:9);
Lys-Ile-Val-Phe-Phe-Ala-$NH_2$ (SEQ ID NO:10);
Lys-Leu-Val-Phe-Phe-Ala-$NH_2$ (SEQ ID NO:11);
Lys-Phe-Val-Phe-Phe-Ala-$NH_2$ (SEQ ID NO:12);
Ala-Phe-Phe-Val-Leu-Lys-$NH_2$ (SEQ ID NO:13);
Lys-Leu-Val-Phe-$NH_2$ (SEQ ID NO:14);
Lys-Ala-Val-Phe-Phe-Ala-$NH_2$ (SEQ ID NO:15);
Lys-Leu-Val-Phe-Phe-$NH_2$ (SEQ ID NO:16);
Lys-Val-Val-Phe-Phe-Ala-$NH_2$ (SEQ ID NO:17);
Lys-Leu-Val-Phe-Phe-Ala-Gln (SEQ ID NO:18);
Lys-Leu-Val-Phe-Phe-Ala-Gln-$NH_2$ (SEQ ID NO:19);
His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-$NH_2$ (SEQ ID NO:20);
His-His-Gln-Lys (SEQ ID NO:23);
and
Gln-Lys-Leu-Val-Phe-Phe-NH2 (SEQ ID NO:24).

16. The labeled conjugate of claim 15, wherein B is selected from the group consisting of Glucose and Phe.

17. The labeled conjugate of claim 15, wherein C is $^{99m}Tc$.

18. A method for the treatment of amyloidosis disorders in a patient, which comprises administering

ART 34 AMDT

to said patient a therapeutically effective amount of a peptide of Formula I as defined in claim 1, 2, 3, 4, 5, 6, 7 or 8.

19. A method for the treatment of amyloidosis disorders in a patient, which comprises administering to said patient a therapeutically effective amount of an antifibrillogenic agent as defined in claim 1, 2, 3, 4, 5, 6, 7 or 8.

20. A composition for the treatment of amyloidosis disorders in a patient, which comprises a therapeutically effective amount of a peptide of Formula I as defined in claim 1, 2, 3, 4, 5, 6, 7 or 8 in association with a pharmaceutically acceptable carrier.

21. A composition for the treatment of amyloidosis disorders in a patient, which comprises a therapeutically effective amount of an antifibrillogenic agent as defined in claim 1, 2, 3, 4, 5, 6, 7 or 8 in association with a pharmaceutically acceptable carrier.

22. A composition for *in vivo* imaging of amyloid deposits, which comprises a therapeutically effective amount of a labeled conjugate as defined in claim 9, 10, 11, 12, 13, 14, 15, 16 or 17 in association with a pharmaceutically acceptable carrier.

23. Use of a peptide of Formula I as defined in claim 1, 2, 3, 4, 5, 6, 7 or 8 for inhibiting amyloidosis and/or for cytoprotection.

ART 34 AMDT

24. Use of an antifibrillogenic agent as defined in claim 1, 2, 3, 4, 5, 6, 7 or 8 for inhibiting amyloidosis and/or for cytoprotection.

25. Use of a labeled conjugate as defined in claim 10, 11, 12, 13, 14, 15, 16 or 17 for *in vivo* imaging of amyloid deposits.

26. Use of a peptide of Formula I as defined in claim 1, 2, 3, 4, 5, 6, 7 or 8 for the manufacture of a medicament for inhibiting amyloidosis and/or for cytoprotection.

27. Use of an antifibrillogenic agent as defined in claim 1, 2, 3, 4, 5, 6, 7 or 8 for the manufacture of a medicament for inhibiting amyloidosis and/or for cytoprotection.

28. Use of a labeled conjugate as defined in claim 10, 11, 12, 13, 14, 15, 16 or 17 for the manufacture of a medicament for *in vivo* imaging of amyloid deposits.

29. A peptide, an isomer thereof, a retro or a retro-inverso isomer thereof or a peptidomimetic thereof, for use in inhibiting amyloidosis and/or for cytoprotection, said peptide having a sequence taken from the β-sheet region of an amyloid protein selected from the group consisting of IAPP and protease resistant prion protein.

30. Use of a peptide as defined in claim 29 for inhibiting amyloidosis and/or for cytoprotection.

ART 34 AMDT

31. Use of a peptide as defined in claim 29 for the manufacture of a medicament for inhibiting amyloidosis and/or for cytoprotection.

32. A composition for inhibiting amyloidosis and/or for cytoprotection, which comprises a therapeutically effective amount of a peptide as defined in claim 31, 30 or 31 in association with a pharmaceutically acceptable carrier.

33. Use of a labeled peptide as defined in claim 29 for the manufacture of a medicament for *in vivo* imaging of amyloid deposits.

34. A process for the preparation of cells suitable for transplantation into a mammal, which cells are capable of forming amyloid deposits, said process comprising contacting the cells *in vitro* with the peptide of Formula I as defined in claim 1 or with the antifibrillogenic compound as defined in claim 1, 2, 3, 4, 5, 6, 7 or 8 for inhibiting amyloid deposit formation.

35. Process according to claim 34, wherein said peptide of Formula I or said antifibrillogenic compound causes breakdown of amyloid deposits, the deposits having been formed by said cells prior to said contact.

36. Process according to claim 34 or 35, in which the cells are cultured in the presence of the peptide of Formula I or the antifibrillogenic compound.

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
C07K 14/47, A61K 38/17, G01N 33/68, A61P 25/28, C12N 5/00, A61K 51/00

A2

(11) International Publication Number: WO 00/68263

(43) International Publication Date: 16 November 2000 (16.11.00)

(21) International Application Number: PCT/CA00/00515

(22) International Filing Date: 4 May 2000 (04.05.00)

(30) Priority Data:
60/132,592 5 May 1999 (05.05.99) US

(71) Applicant *(for all designated States except US)*: NEUROCHEM, INC. [CA/CA]; 7220 Frederick–Banting, Suite 100, Montréal, Québec H4S 2A1 (CA).

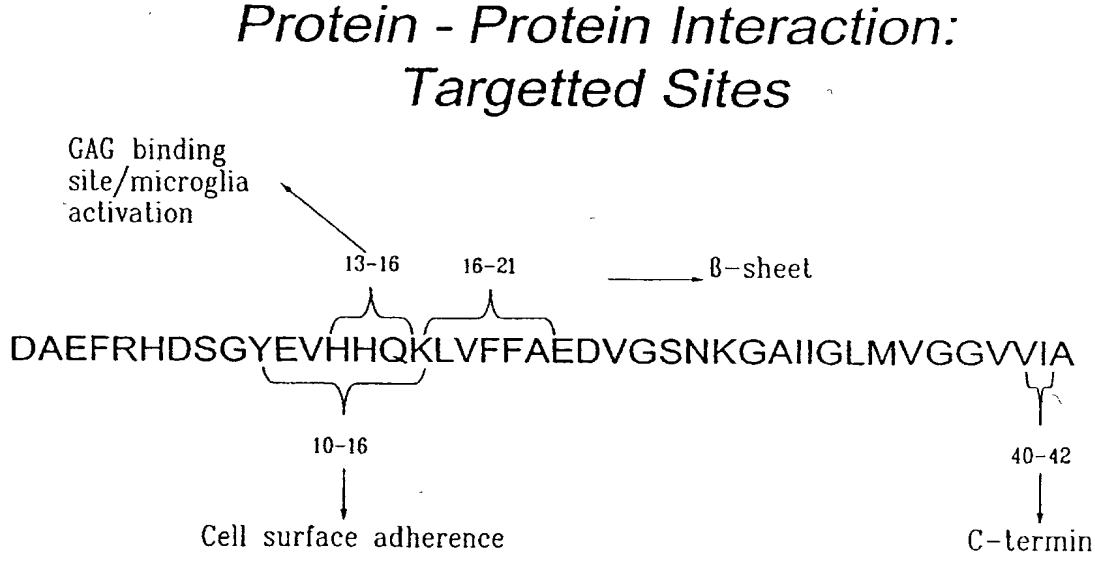
(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: CHALIFOUR, Robert [CA/CA]; 332 Place Ladouceur, Ile Bizard, Québec H9C 1T4 (CA). GERVAIS, Francine [CA/CA]; 1003 Bellevue, Ile Bizard, Québec H9C 2X5 (CA). GUPTA, Ajay [CA/CA]; 255 St–Louis, Pointe–Claire, Québec H9R 5L6 (CA).

(74) Agents: MURPHY, Kevin, P. et al.; Swabey Ogilvy Renault, Suite 1600, 1981 Mcgill College Avenue, Montréal, Québec H3A 2Y3 (CA).

(81) Designated States: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

**Published**
*Without international search report and to be republished upon receipt of that report.*

(54) Title: STEREOSELECTIVE ANTIFIBRILLOGENIC PEPTIDES AND PEPTIDOMIMETICS THEREOF




(57) Abstract

The present invention relates to antifibrillogenic agents for inhibiting amyloidosis and/or for cytoprotection for the treatment of amyloidosis disorders. These agents include peptides, isomers thereof and peptidomimetic compounds thereof. These agents comprise a peptide having a sequence identified from the glycosaminoglycan (GAG) binding region and the prot–prot interaction region of the amyloid protein. The peptide has at least one [D] amino acid isomer substitution. The invention also relates to the peptide bound to a label for *in vivo* imaging of amyloid deposits.

# Protein - Protein Interaction: Targetted Sites

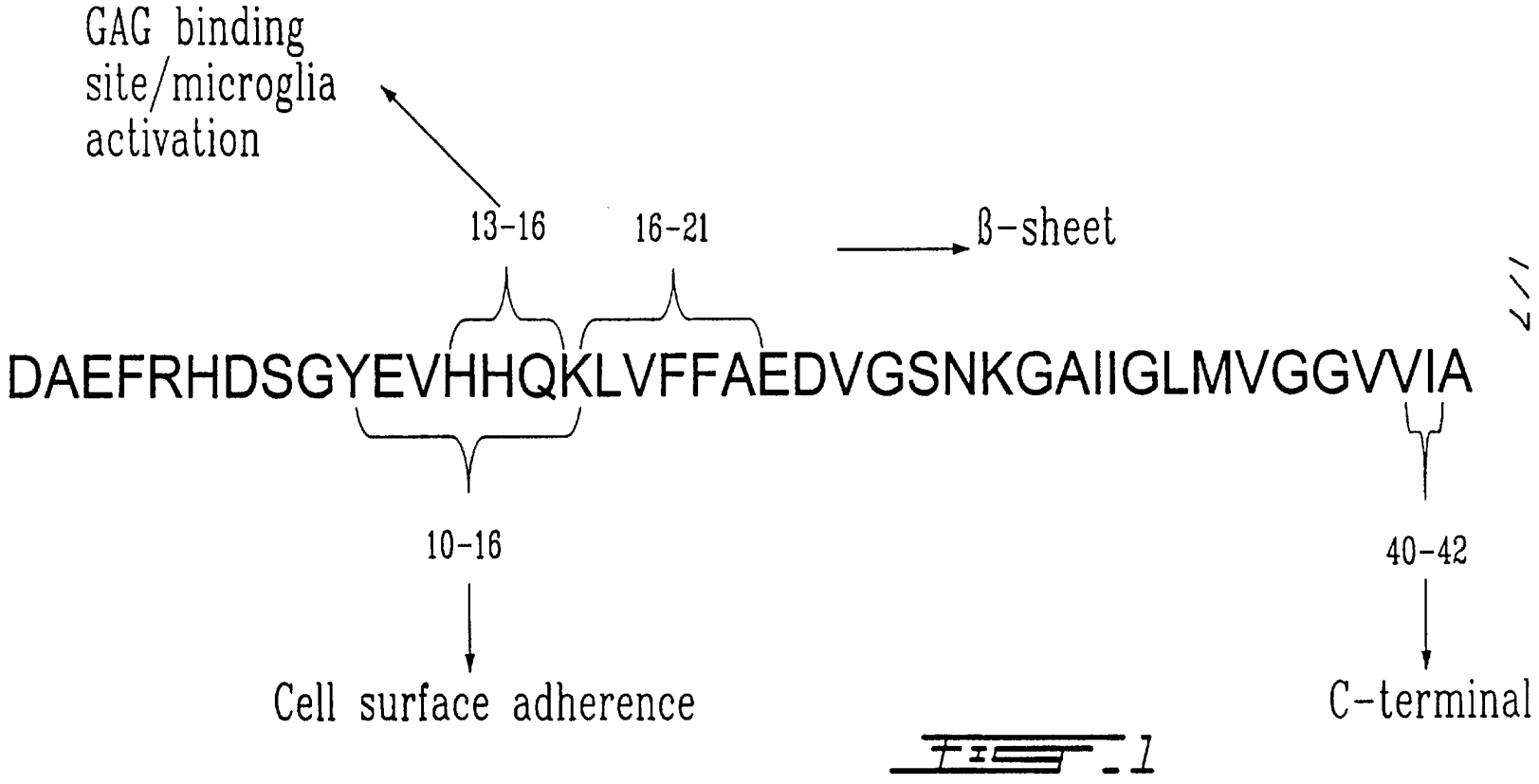



Fig. 1



10/009122

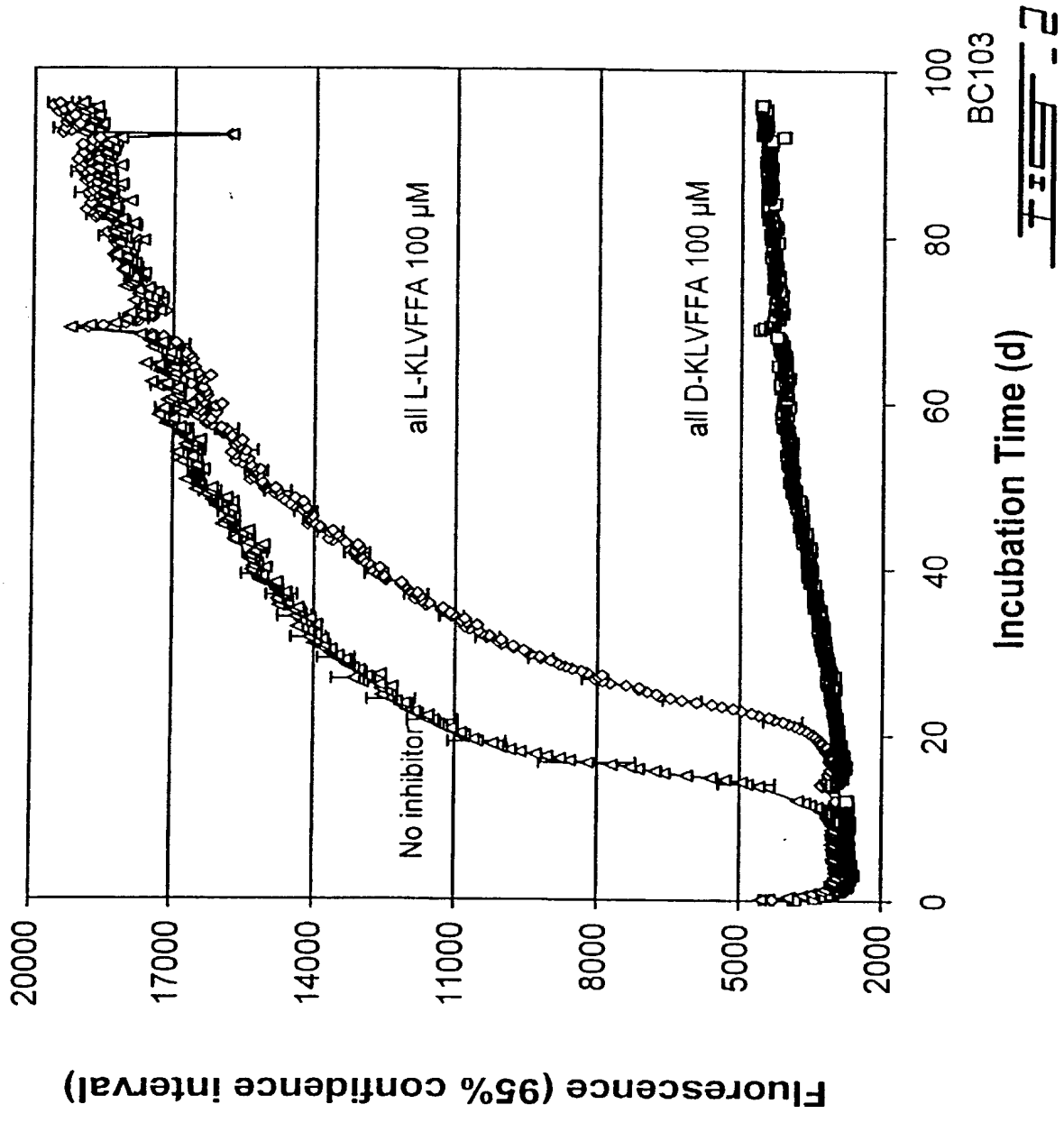
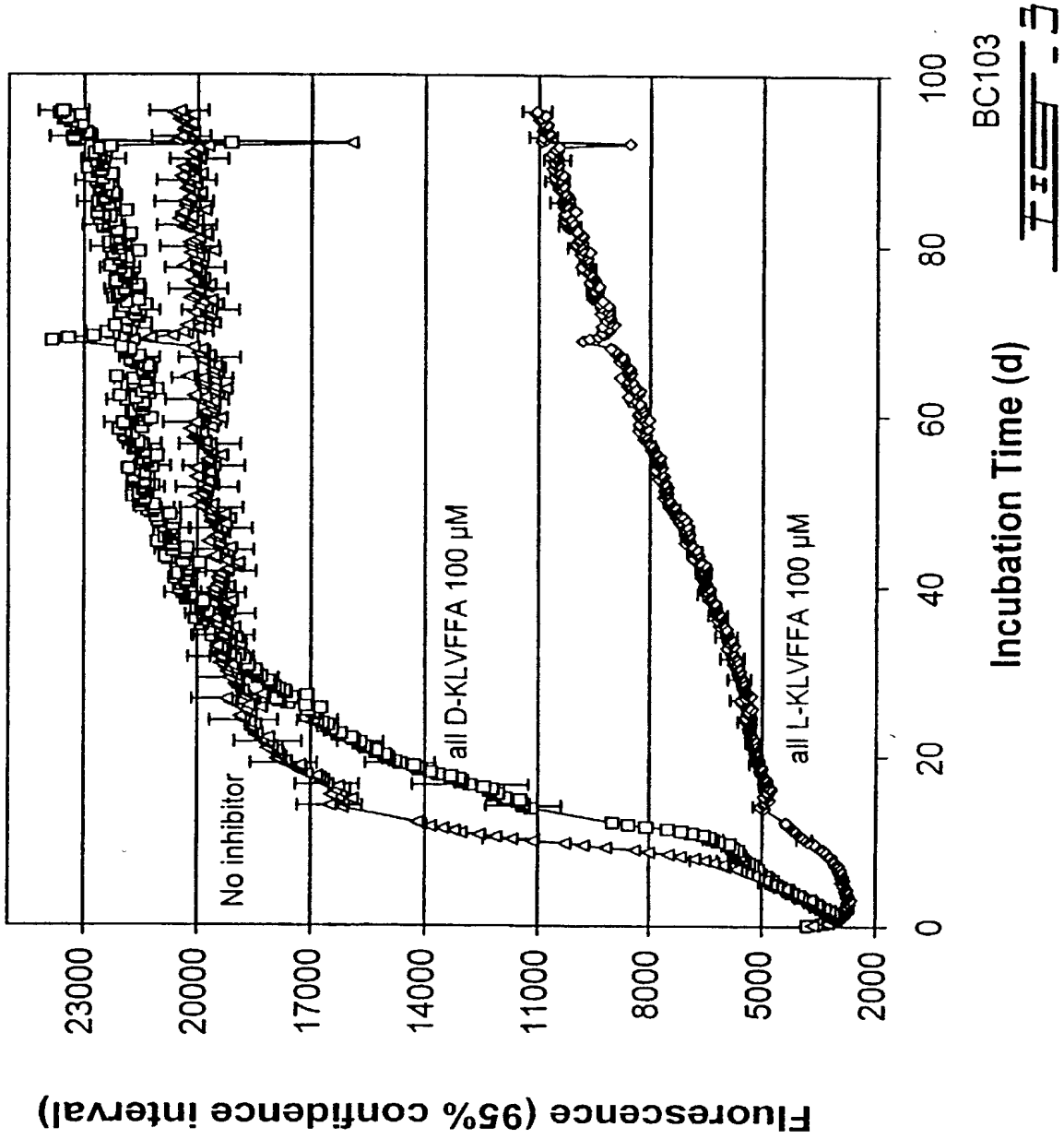

Fluorescence (95% confidence interval)
20000
17000
14000
11000
8000
5000
2000
0
20
40
60
80
100
Incubation Time (d)
BC103
all L-KLVFFA 100 µM
all D-KLVFFA 100 µM
No inhibitor

Fig - 2


BC103
Incubation Time (d)
0
20
40
60
80
100
Fluorescence (95% confidence interval)
2000
5000
8000
11000
14000
17000
20000
23000
No inhibitor
all D-KLVFFA 100 µM
all L-KLVFFA 100 µM

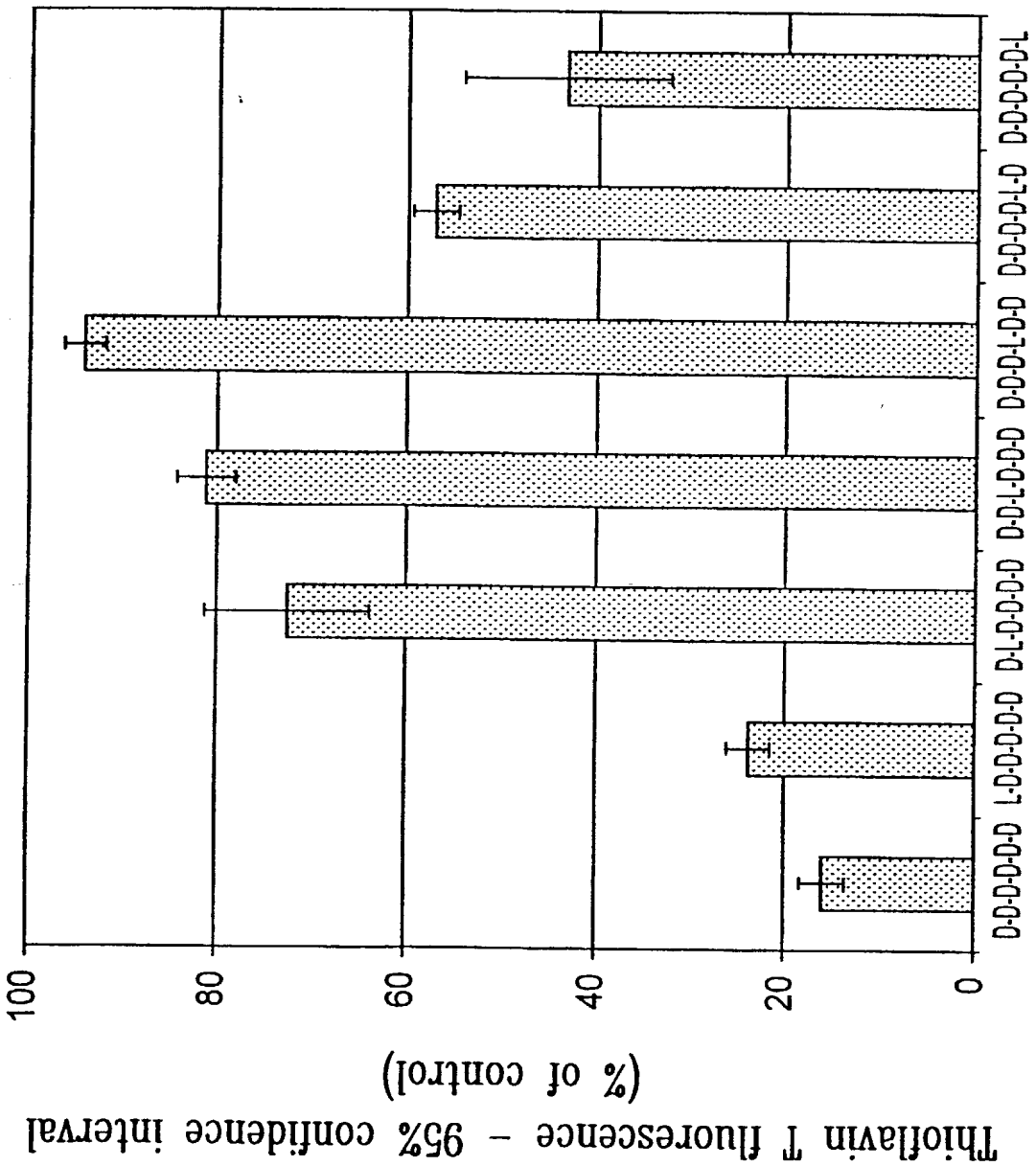

Thioflavin T fluorescence – 95% confidence interval
(% of control)
100
80
60
40
20
0
D-D-D-D-D-D L-D-D-D-D-D D-L-D-D-D-D D-D-L-D-D-D D-D-D-L-D-D D-D-D-D-L-D D-D-D-D-D-L
position of L-amino acid insertion
BC130

Fig. 4

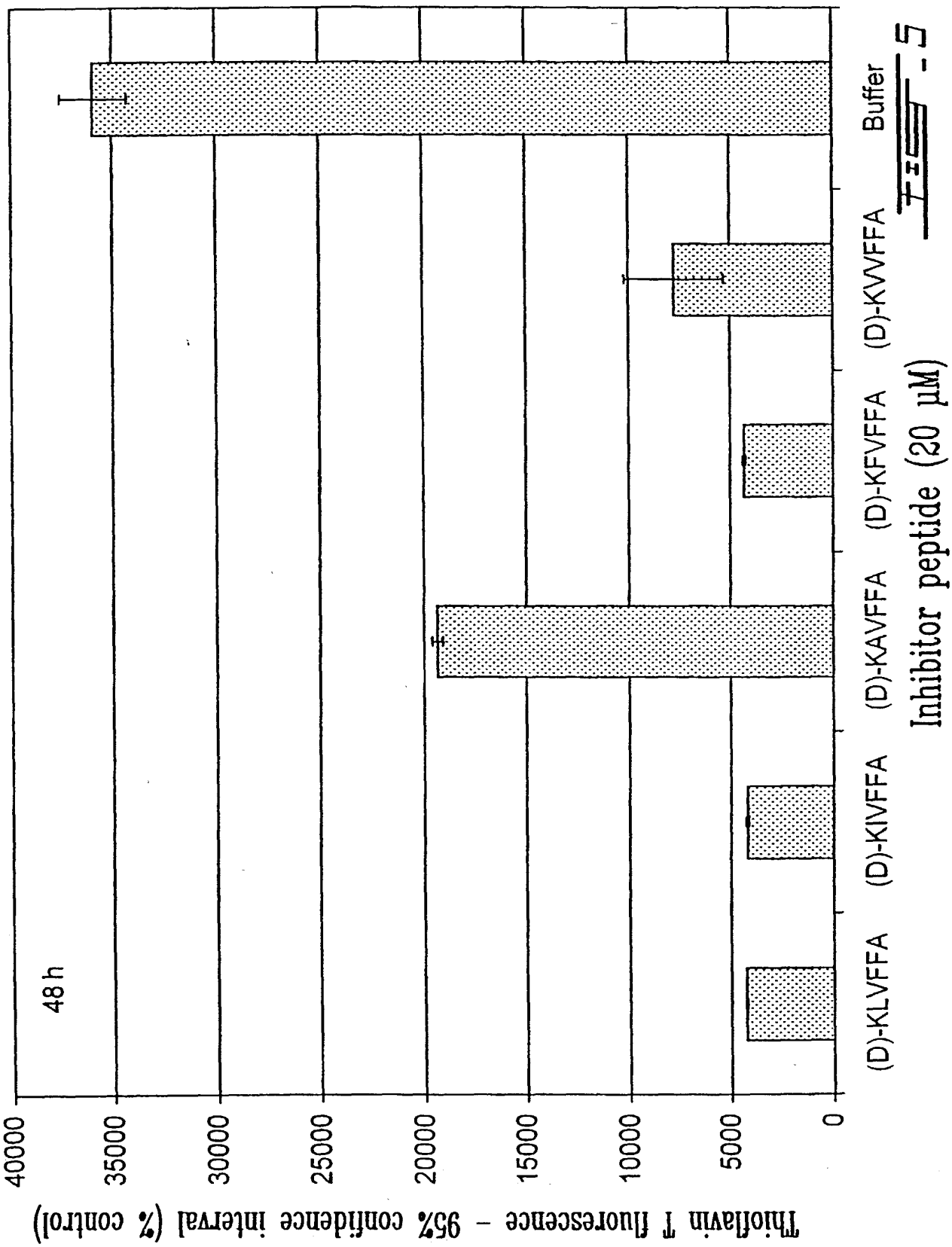

Thioflavin T fluorescence – 95% confidence interval (% control)
40000
35000
30000
25000
20000
15000
10000
5000
0
48 h
(D)-KLVFFA
(D)-KIVFFA
(D)-KAVFFA
(D)-KFVFFA
(D)-KVVFFA
Buffer
Inhibitor peptide (20 µM)

Fig - 5

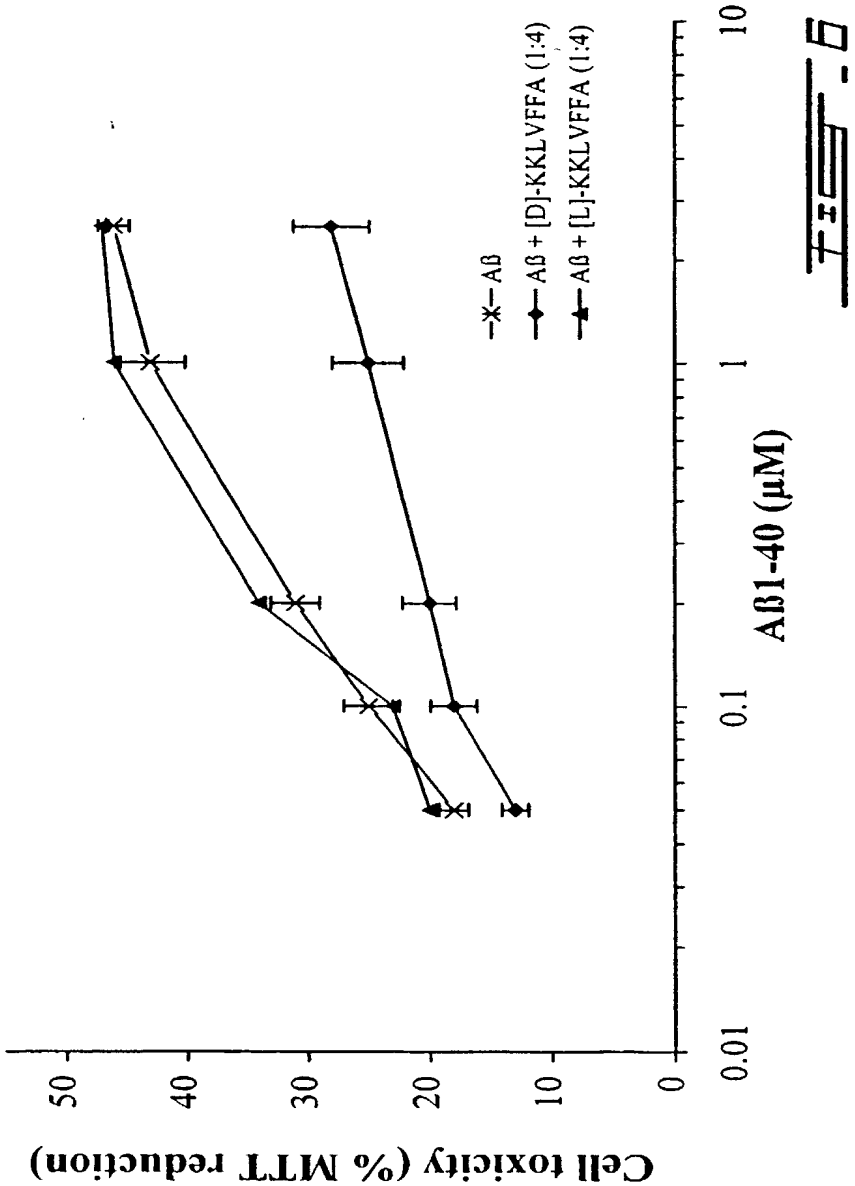

Cell toxicity (% MTT reduction)
0
10
20
30
40
50
0.01
0.1
1
10
Aß1-40 (µM)
Aß
Aß + [D]-KKLVFFA (1:4)
Aß + [L]-KKLVFFA (1:4)

Fig - 6

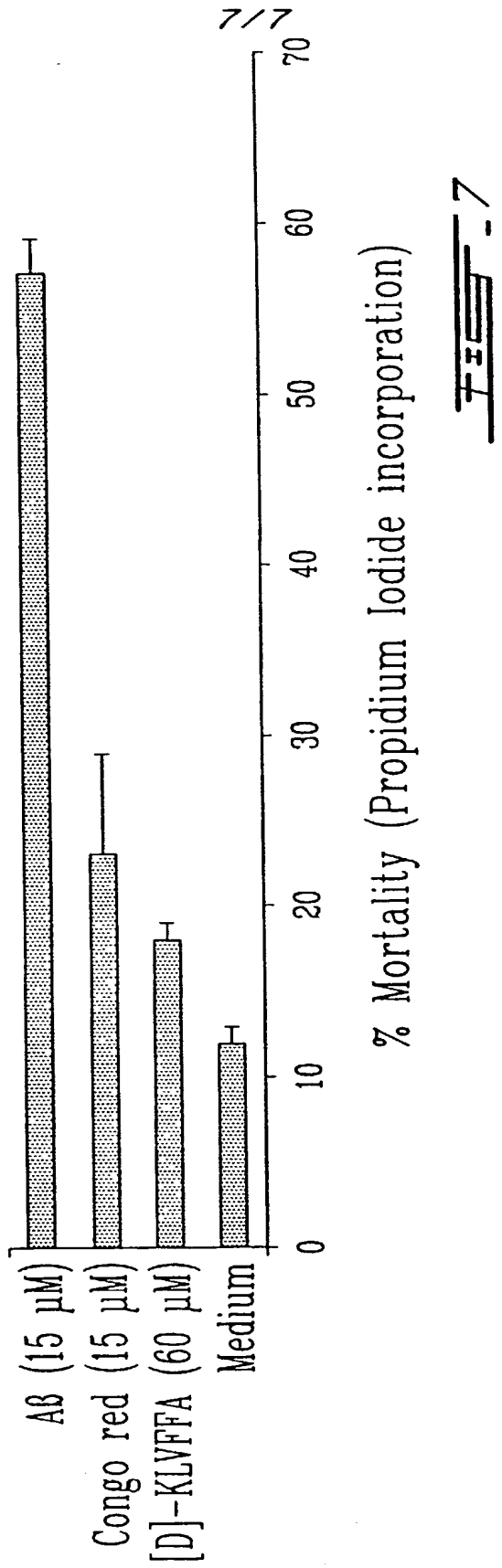

7/7
% Mortality (Propidium Iodide incorporation)
0
10
20
30
40
50
60
70
Aß (15 µM)
Congo red (15 µM)
[D]-KLVFFA (60 µM)
Medium

Fig. 7

Express Mail Label No.: ET318110804US
Date of Deposit: June 18, 2002

Attorney Docket No. 14445-504NATL

# COMBINED DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am an original, first and joint inventor of the subject matter which is claimed and for which a utility patent is sought on the invention entitled:

## STEREOSELECTIVE ANTIFIBRILLOGENIC PEPTIDES AND PEPTIDOMIMETICS THEREOF

the specification of which:

☒ was filed on 04 May 2000 as a PCT application designating the United States, and was assigned PCT/CA00/00515 and entered the National Phase in the United States on November 5, 2001. The application has been assigned Serial No. 10/009,122, and bears Attorney Docket Number 14445-504.

☐ is attached hereto.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56.

☐ I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT International application designating at least one country other than the United States listed below and have also identified below any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

| **Appln. Number** | **Country** *(if PCT, so indicate)* | **Filing Date** *(dd/mm/yy)* | **Priority Claimed** | |
|---|---|---|---|---|
| | | | **Yes** | **No** |
| | | | ☐ | ☐ |
| | | | ☐ | ☐ |
| | | | ☐ | ☐ |

☐ I hereby claim the benefit under Title 35, United States Code, § 119(e) or §120 of any United States application(s), or §365(c) of any PCT International application(s) designating the United States of America listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

| **Application No.** *(U.S.S.N.)* | **Filing Date** *(dd/mm/yy)* | **Status** *(Patented, Pending, Abandoned)* |
|---|---|---|
| | | |
| | | |

PCT International Applications designating the United States:

| **PCT International Application No.** | **PCT Filing Date** | **Status** |
|---|---|---|
| PCT/CA00/00515 | 04 May 2000 | Pending |

I hereby appoint the following attorneys and/or agents to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

| **Attorney or Agent** | **Registration No.** | **Attorney or Agent** | **Registration No.** |
|---|---|---|---|
| Kevin Ainsworth | 39,586 | Brian P. Hopkins | 42,669 |
| Ingrid Beattie | 42,306 | Shane Hunter | 41,858 |
| William Belanger | 40,509 | David E. Johnson | 41,874 |
| Charles E. Bell | 48,128 | Christina Karnakis | 45,899 |
| Naomi Biswas | 38,384 | Kristin E. Konzak | 44,848 |
| Bradford C. Blaise | 47,429 | Cynthia Kozakiewicz | 42,764 |
| Sean M. Coughlin | 48,593 | Barry Marenberg | 40,715 |
| David F. Crosby | 36,400 | A. Jason Mirabito | 28,161 |
| Christopher J. Cuneo | 42,450 | Michel Morency | 50,183 |
| Brett N. Dorny | 35,860 | Matthew Pavao | 50,572 |
| Marianne Downing | 42,870 | Carol H. Peters | 45,010 |
| Ivor R. Elrifi | 39,529 | David Poirier | 43,007 |
| Heidi A. Erlacher | 45,409 | Michael Renaud | 44,299 |
| Eugene A. Feher | 33,171 | Brian Rosenbloom | 41,276 |
| John M. Garvey | 37,833 | Robert J. Sayre | 42,124 |
| James G. Gatto | 32,694 | C. Eric Schulman | 43,350 |
| Richard Gervase | 46,725 | Gregory J. Sieczkiewicz | 48,223 |
| Matthew J. Golden | 35,161 | Janine M. Susan | 46,119 |
| Sonia K. Guterman | 44,729 | Howard Susser | 33,556 |
| John Harre | 37,345 | Nicholas P. Triano, III | 36,397 |
| Paul J. Hayes | 28,307 | | |

all of MINTZ, LEVIN, COHN, FERRIS, GLOVSKY AND POPEO PC, One Financial Center, Boston, Massachusetts 02111, as Applicant's attorneys with full power of substitution and revocation to take any and all action necessary with regard to the above-identified patent.

Address all telephone calls to Barry J. Marenberg, Esq., at telephone number 617/348-3099.

Address all correspondence to:

Michel Morency, Esq.
Mintz, Levin, Cohn, Ferris, Glovsky and Popeo, P.C.
One Financial Center
Boston, Massachusetts 02111

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or patent issued thereon.

Robert Chalifour — May 13th, 2002
Inventor's Signature — Date
Full Name of Inventor: Robert CHALIFOUR
Citizenship: Canada
Residence: 332 Place Ladouceur, Ile Bizard, Quebec H9C 1T4 Canada
Post Office Address: Same as Above

Francine Gervais — May 13th, 2002
Inventor's Signature — Date
Full Name of Inventor: Francine GERVAIS
Citizenship: Canada
Residence: 1003 Bellevue, Ile Bizard, Quebec H9C 2X5 Canada
Post Office Address: Same as Above

A. Gupta — May 07th, 2002
Inventor's Signature — Date
Full Name of Inventor: Ajay GUPTA
Citizenship: Canada
Residence: 255 St. Louis, Pointe Claire, Quebec H9R 5L6 Canada
Post Office Address: Same as Above

SEQUENCE LISTING

<110> NEUROCHEM INC.
CHALIFOUR, Robert
GERVAIS, Francine
GUPTA, Ajay

<120> STEREOSELECTIVE ANTIFIBRILLOGENIC PEPTIDES AND PEPTIDOMIMETICS THEREOF

<130> 14228-1PCT

<150> US 60/132,592
<151> 1999-05-05

<160> 24

<170> FastSEQ for Windows Version 3.0

<210> 1
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> peptide having antifibrillogenic activity and/or cytoprotection

<400> 1
Lys Ile Val Phe Phe Ala
1 5

<210> 2
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> peptide having antifibrillogenic activity and/or cytoprotection

<400> 2
Lys Lys Leu Val Phe Phe Ala
1 5

<210> 3
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> peptide having antifibrillogenic activity and/or cytoprotection

<400> 3
Lys Leu Val Phe Phe Ala

1 5

<210> 4
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> peptide having antifibrillogenic activity and/or cytoprotection

<400> 4
Lys Phe Val Phe Phe Ala
1 5

<210> 5
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> peptide having antifibrillogenic activity and/or cytoprotection

<400> 5
Ala Phe Phe Val Leu Lys
1 5

<210> 6
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> peptide having antifibrillogenic activity and/or cytoprotection

<400> 6
Lys Leu Val Phe
1

<210> 7
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> peptide having antifibrillogenic activity and/or cytoprotection

<400> 7
Lys Ala Val Phe Phe Ala
1 5

<210> 8
<211> 5
<212> PRT

<213> Artificial Sequence

<220>
<223> peptide having antifibrillogenic activity and/or cytoprotection

<400> 8
Lys Leu Val Phe Phe
1 5

<210> 9
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> peptide having antifibrillogenic activity and/or cytoprotection

<400> 9
Lys Val Val Phe Phe Ala
1 5

<210> 10
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> peptide having antifibrillogenic activity and/or cytoprotection

<221> AMIDATION
<222> (6)...(6)

<400> 10
Lys Ile Val Phe Phe Ala
1 5

<210> 11
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> peptide having antifibrillogenic activity and/or cytoprotection

<221> AMIDATION
<222> (6)...(6)

<400> 11
Lys Leu Val Phe Phe Ala
1 5

<210> 12
<211> 6

<212> PRT
<213> Artificial Sequence

<220>
<223> peptide having antifibrillogenic activity and/or cytoprotection

<221> AMIDATION
<222> (6)...(6)

<400> 12
Lys Phe Val Phe Phe Ala
1 5

<210> 13
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> peptide having antifibrillogenic activity and/or cytoprotection

<221> AMIDATION
<222> (6)...(6)

<400> 13
Ala Phe Phe Val Leu Lys
1 5

<210> 14
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> peptide having antifibrillogenic activity and/or cytoprotection

<221> AMIDATION
<222> (4)...(4)

<400> 14
Lys Leu Val Phe
1

<210> 15
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> peptide having antifibrillogenic activity and/or cytoprotection

<221> AMIDATION
<222> (6)...(6)

<400> 15
Lys Ala Val Phe Phe Ala
1 5

<210> 16
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> peptide having antifibrillogenic activity and/or cytoprotection

<221> AMIDATION
<222> (5)...(5)

<400> 16
Lys Leu Val Phe Phe
1 5

<210> 17
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> peptide having antifibrillogenic activity and/or cytoprotection

<221> AMIDATION
<222> (6)...(6)

<400> 17
Lys Val Val Phe Phe Ala
1 5

<210> 18
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> peptide having antifibrillogenic activity and/or cytoprotection

<400> 18
Lys Leu Val Phe Phe Ala Gln
1 5

<210> 19
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> peptide having antifibrillogenic activity and/or

cytoprotection

<221> AMIDATION
<222> (7)...(7)

<400> 19
Lys Leu Val Phe Phe Ala Gln
1 5

<210> 20
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> peptide having antifibrillogenic activity and/or cytoprotection

<221> AMIDATION
<222> (9)...(9)

<400> 20
His His Gln Lys Leu Val Phe Phe Ala
1 5

<210> 21
<211> 3
<212> PRT
<213> Artificial Sequence

<220>
<223> peptide having antifibrillogenic activity and/or cytoprotection

<400> 21
Asp Asp Asp
1

<210> 22
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> peptide having antifibrillogenic activity and/or cytoprotection

<400> 22
Lys Val Asp Asp Gln Asp
1 5

<210> 23
<211> 4
<212> PRT
<213> Artificial Sequence

<220>

<223> peptide having antifibrillogenic activity and/or cytoprotection

<400> 23
His His Gln Lys
1

<210> 24
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> peptide having antifibrillogenic activity and/or cytoprotection

<221> AMIDATION
<222> (6)...(6)

<400> 24
Gln Lys Leu Val Phe Phe
1 5